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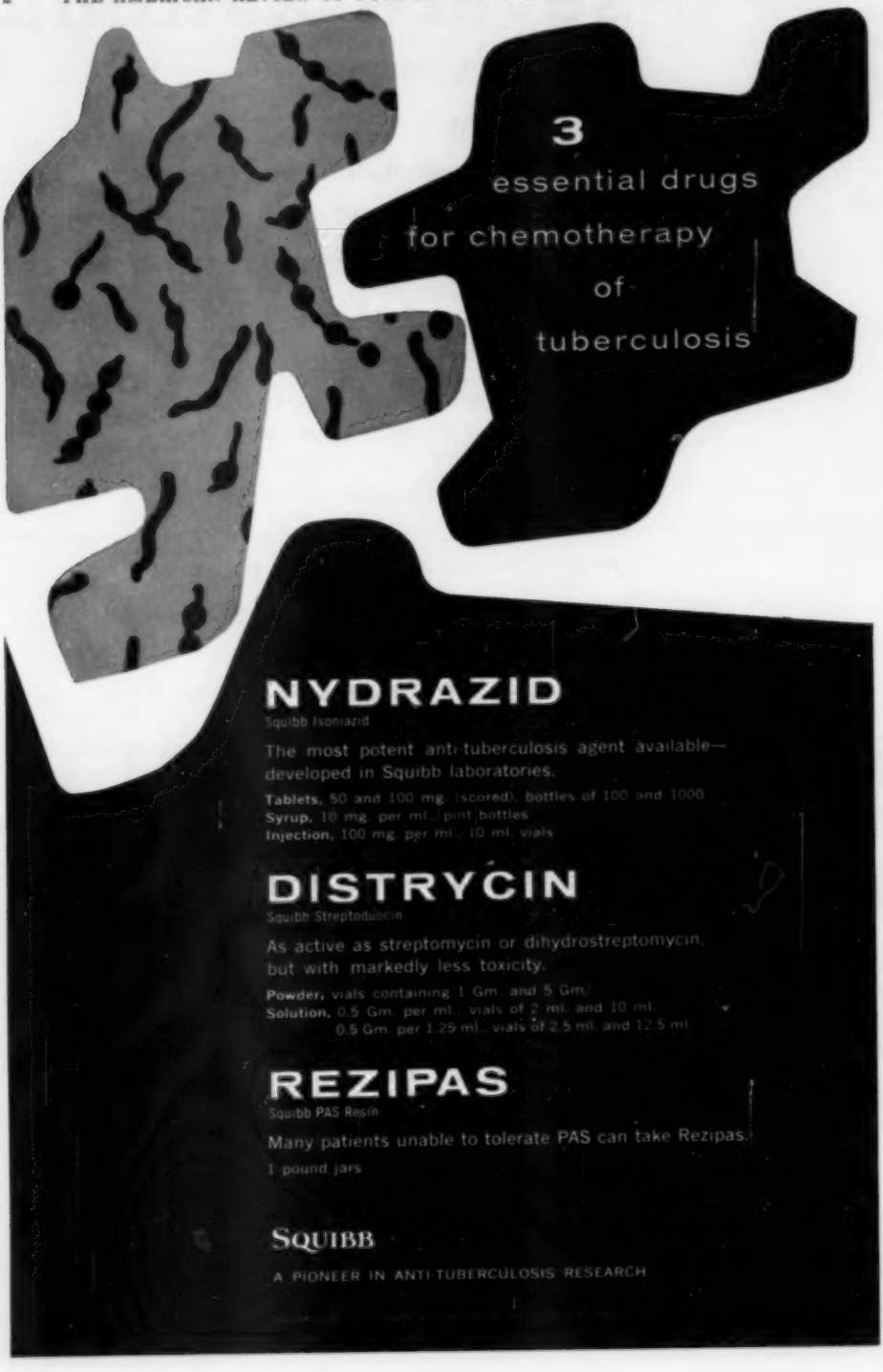
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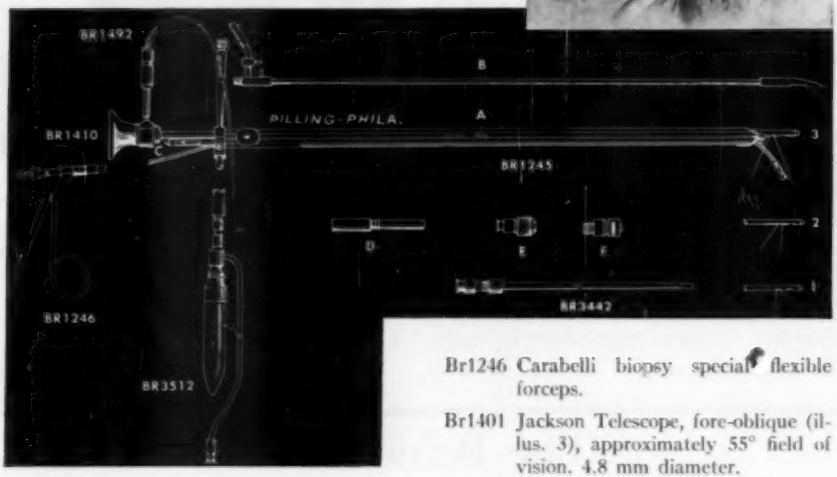

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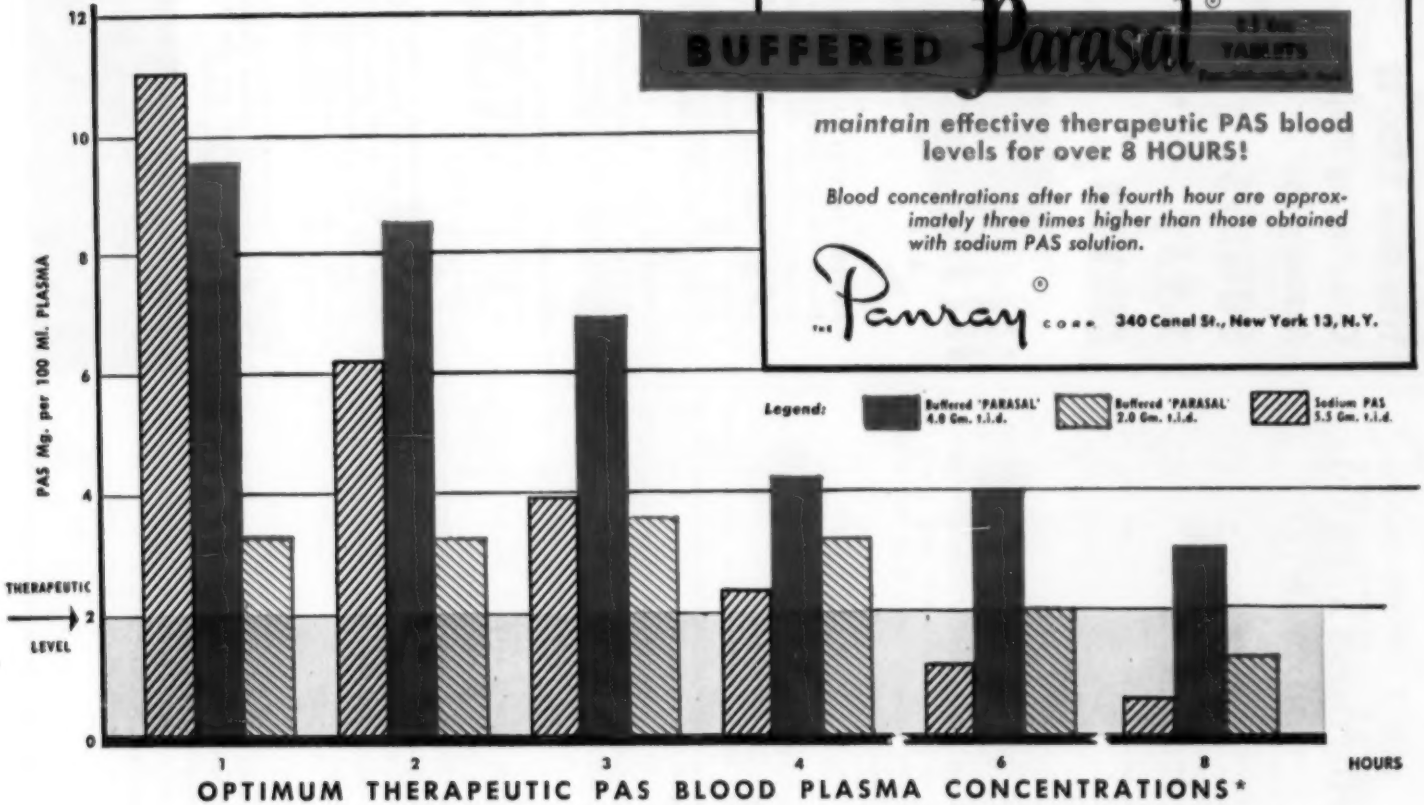

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 75 *January, 1957* NUMBER 1

MINIMAL PULMONARY TUBERCULOSIS IN MILITARY PERSONNEL: WORLD WAR II[1,2,3]

JAMES J. WARING[4] AND WILLIAM H. ROPER[5]

(Received for publication September 12, 1955)

Introduction

The mobilization and maintenance of the Army of the United States in World War II, with initial physical examination of all accepted military personnel and subsequent medical control and hospitalization as necessary, provided an exceptionally valuable opportunity for studying the fate of tuberculous lesions. The medical provisions of the mobilization regulations, permitting acceptance of men with small densely scarred lesions, and the occurrence of active tuberculosis in the military forces as a result of failure of detection on entry or new acquisition of the disease in the course of service, made it possible to study in army hospitals, or in a known military environment, the progress of tuberculous lesions that became manifest under conditions ranging from sedentary occupations to the extreme physical strains of military combat.

The study here described was set up to take advantage of this unusual opportunity. It is believed that a report of its findings will be of interest and value at the present time for comparison with currently accumulating data on the course of pulmonary tuberculosis of similar character as affected by methods of treatment (specific chemotherapy) not available at the time of this study.

Plan of Study

A research organization was established that would permit observation over a period of years of a group of a thousand men and women with pulmonary

[1] This study has been made by the section for research on minimal tuberculosis, U. S. Army Medical Research and Development Board, Fitzsimons Army Hospital, and the University of Colorado, School of Medicine, Denver, Colorado.

[2] The study was initiated in January, 1944, with Dr. James J. Waring as Responsible Investigator, Colonel George F. Aycock and Colonel Esmond R. Long as consultants, and Lieutenant Colonel William H. Roper as Director of the Section.

[3] The study was aided by a grant from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association.

[4] Present address: Colorado Foundation for Research in Tuberculosis, 4200 East Ninth Avenue, Denver 20, Colorado.

[5] Present address: Division of Tuberculosis Control, New York State Department of Health, 84 Holland Avenue, Albany 8, New York.

tuberculosis in the minimal stage at the time of its first detection, or with reliable records furnishing objective evidence of its presence in that stage at some previous period, e.g., in an army induction station examination in which the lesion was overlooked. A group of approximately this size was selected by procedures indicated in the following paragraphs, and a system of follow-up observation for a period of three years or more was set up in order to obtain data for analysis with respect to factors that might have a significant bearing on the course of the disease.

Because of the nature of the material studied, the observations were oriented on a military basis. In essence, however, they apply equally well to tuberculosis in a nonmilitary environment, for factors such as age of the subjects, the state of activity of tuberculosis when first detected, chronologic sequences in its progression and regression, and the frequency of relapse are all of general as well as military significance.

In gathering data in the military environment, inquiry was directed to: (*1*) the course of the illness, in so far as it could be determined in retrospect, between acceptance for military service and the initial diagnosis; (*2*) evidence of tuberculosis prior to military service; (*3*) the course of the disease with reference to its status as observed in the first roentgenograms available in the military environment; (*4*) the manner of onset and the course and severity of the disease in relation to the military factors of rank, occupation and duties, places of service, and physical stress and strain; (*5*) the course of the disease after it became manifest, with particular reference to its manner of onset, the pathologic character of its lesions, and the length and nature of treatment; and, finally, (*6*) the frequency and severity of relapse in relation to all of the above-named factors and influences.

The selection of patients for investigation was based on the desire to follow the course of apparently active lesions, on the one hand, and to study the stability of supposedly inactive lesions, on the other hand, in persons subjected originally to a wide variety of environmental conditions and subsequently treated in hospitals or observed on an ambulant basis. The organization of the army medical services facilitated the fulfillment of these objectives. Many patients with active tuberculosis, with comprehensive records of the course of their disease subsequent to its recognition, were available for study in army hospitals, and the conditions of medical follow-up in the army after a period of medical observation were such that it was possible to make periodic studies of men and women returned to duty after a diagnosis of inactive or arrested disease.

Selection of patients: The selection of patients for study was made by one of the investigators (WHR) in the course of visits at thirty-six army hospitals. These included three tuberculosis centers (Fitzsimons Army Hospital, Bruns General Hospital, and Moore General Hospital) and thirty-three other military hospitals in other medical centers or large training camps. At the three tuberculosis centers, which received large numbers of patients referred from elsewhere, cases of tuberculosis of minimal extent were so numerous that a lottery system was employed to ensure a randomly selected representative sample, unweighted with respect to individual factors. The names of all

patients qualified for inclusion were arranged in chronologic order by date of hospital admission, and every fifth name was chosen. At the thirty-three other hospitals, which received cases directly from army field or service installations, cases were discovered with such infrequency that it was possible to include in the study without bias all patients discovered at those hospitals during the period of selection of patients. In the army installations served by these thirty-three hospitals, cases of active and inactive tuberculosis were detected from time to time in the course of routine examination, i.e., for admission to officer candidate school, or through examinations made because of symptoms of pulmonary abnormality. It was believed by the investigators and consultants that the purposes of the study, as outlined above, would be served best if approximately one-third of the group selected consisted of cases apparently inactive and two-thirds of cases apparently active when first discovered. Under the plan of study, as noted above, all cases were to be followed for three years or more from the time of first observation for tuberculosis.

The initial selection of cases was carried out over a period of two and a half years from January, 1944, to June, 1946, and all patients included in the report were followed for at least three years, the average for the group slightly exceeding four years (51.2 person-months after the first interview in the course of the study).

How effectively the aims of the selection were satisfied may be determined from the following figures. The total number of patients studied was 967, comprised of 922 men and 45 women; 880 were white persons and 87 Negroes or other non-whites. Of the 967 persons, approximately two-thirds (625) had tuberculosis which, in the opinion of the investigators, satisfied the criteria (*see* below) at initial observation for active disease; and one-third (342), the criteria set up for apparently inactive or arrested disease.

In all, 391 persons were first examined by physicians of the Army Research Section at Fitzsimons Army Hospital, 205 at Bruns General Hospital and Moore General Hospital, and 371 at other military hospitals. In 594 of the 967 persons selected for study, the tuberculous disease (active or apparently inactive) was first recognized by routine roentgenograms, by roentgenograms made upon admission to the hospital for other causes, or, for example, when applying for admission to officer candidate school. None of these patients had premonitory symptoms. The remaining 373 patients had cough, chest pains, fatigability, or other symptoms which led to roentgenograms of the chest and to the diagnosis. Actually, none of these patients was "sick." In most instances their symptoms were mild and doubtless, in civilian life, would have been considered inconsequential.

The method of selecting patients for study is believed to have yielded a valid random sample of minimal pulmonary tuberculosis in the army. For example, 4.7 per cent of the patients included in this project were female, compared with 5.0 per cent of patients with minimal disease in the entire United States Army, as reported to the office of the Surgeon General for the same period as that covered by this study. Similarly, commissioned officers comprised 19.3 per cent of the patients analyzed by the Army Research Section, compared with 15.8 per cent of patients in the army as a whole.

The racial composition of patients in the two groups cannot be compared satisfactorily because of minor differences in classification between this study and the army's official records. In the present study, 880 (91 per cent) of the group were classified as white.

Persons who came from truly urban communities numbered 433, those from truly rural areas numbered 279. The remaining 255 had resided in what might be termed rural-urban communities. Of the 967 persons in this study, 300 (31 per cent) gave histories of exposure to tuberculosis in pre-military life.

Comparison of the research group with the total census of cases of active minimal or

TABLE 1

PATIENTS WITH ACTIVE AND INACTIVE MINIMAL PULMONARY TUBERCULOSIS IN THE STUDY GROUP AND IN THE ENTIRE UNITED STATES ARMY, CLASSIFIED BY AGE GROUP: JANUARY 1, 1944, TO JUNE 30, 1946

Age Group	Active Cases				Inactive Cases			
	In Study Group		In U. S. Army		In Study Group		In U. S. Army	
	Number	Per Cent Distribution	Number	Per Cent Distribution	Number	Per Cent Distribution	Number	Per Cent Distribution
All age groups	625	100.0	4,070	100.0	342	100.0	7,045	100.0
Less than 20 years	13	2.1	183	4.5	17	5.0	218	3.1
20–24 years	169	27.0	1,123	27.6	88	25.7	1,599	22.7
25–29 years	206	33.0	1,209	29.7	102	29.8	2,015	28.6
30–34 years	131	21.0	810	19.9	75	21.9	1,451	20.6
35–39 years	79	12.6	505	12.4	43	12.6	1,029	14.6
40 years and older	27	4.3	240	5.9	17	5.0	733	10.4
Median age	28.2		28.0		28.3		29.2	

inactive tuberculosis for the over-all army indicated a close correspondence with respect to officer and enlisted personnel in the two groups. Of the 967 persons in the study group, 187 were commissioned officers and 780 enlisted personnel. Approximately 40 per cent were in the Ground Forces of the Army, 40 per cent in the Service Forces, and 20 per cent in the Air Forces.

The age distributions of active and apparently inactive minimal cases included in this project are compared, in table 1, with similar data for *all active minimal cases* of pulmonary tuberculosis and *all inactive or arrested cases* in the army as a whole during the same period. Data for inactive cases in the medical division of the office of the Surgeon General are not classified specifically as to stage of disease; however, the disease must have been minimal in extent in the vast majority of such cases.

The similarity in age distribution of the two major groups is immediately evident in table 1. It seems much more pronounced, however, when it is realized that the median age of *all* patients included in the study was 28.2 years, compared with 28.8 years for *all* patients in the army as a whole. If the comparison is restricted to active cases, the median age of patients in the study group is almost identical with that of patients in the entire United States Army. By far the highest median age is that shown for inactive disease in patients in the army as a whole (29.2 years).

Criteria for diagnosis: The diagnosis of active or inactive tuberculosis was made at the time of examination of the first chest roentgenogram taken in military service which showed evidence of tuberculosis, or within six months of that time in cases requiring this period for evidence of stability. It was recognized that roentgenographic distinction between active and inactive disease is very often uncertain, that different radiologists might interpret a given film differently, and that an individual radiologist might not make the same diagnosis on successive readings of the same film. Hence the diagnosis was inevitably an arbitrary one representing simply the best judgment of the physicians in the Army Research Section. Long and Jablon (1), in their "evaluation of X-ray screening," stress the unreliability of single readings of roentgenograms of the chest and the great importance of dual reading with subsequent conferences on disagreements.

TABLE 2

TUBERCULIN-POSITIVE PATIENTS GIVEN OTHER INTRACUTANEOUS SENSITIVITY TESTS AFTER ADMISSION TO THE ARMY, CLASSIFIED BY TYPE OF TEST AND RESULT

Type of Test and Result	Tuberculin-Positive Patients					
	Total	Per Cent Distribution	Given First Strength		Given Second Strength	
			Number	Per Cent Distribution	Number	Per Cent Distribution
All types	967	100.0	783	100.0	184	100.0
Coccidioidin negative, histoplasmin negative	663	68.6	535	68.3	128	69.6
Coccidioidin negative, histoplasmin positive	210	21.7	181	23.1	29	15.8
Coccidiodin positive, histoplasmin negative	43	4.4	26	3.3	17	9.2
Coccidioidin positive, histoplasmin positive	51	5.3	41	5.2	10	5.4

A definition of terms follows:

1. *Active disease.* The criteria for the diagnosis of *active* minimal pulmonary tuberculosis were:

a. Roentgenogram.[6] "Characteristic" soft, mottled or nodular, unstable lesion without evident cavity, usually in the apex of the upper lobe of the lung, which failed to clear completely in six weeks or more, and for which there was no other adequate explanation. The amount of involvement did not exceed the National Tuberculosis Association 1950 standards of classification of "minimal" disease.

b. Tuberculin skin test. *Positive;* some also had positive coccidioidin and/or positive histoplasmin skin tests.

c. Tubercle bacilli, sputum and/or gastric washings, positive preferable but not essential.

2. *Inactive disease.* The criteria for the diagnosis of *inactive* minimal pulmonary tuberculosis were:

a. "Characteristic" roentgenographic shadows of fibrous, fibronodular, fibrocalcific, or fibronodular-calcific lesions, not exceeding National Tuberculosis Association 1950 standards, usually in the upper portion of a lobe, showing no change over a period of at least six months, and with no other adequate explanation.

b. Tuberculin skin test. *Positive*, with no reaction to coccidioidin and/or histoplasmin in practically all cases.

c. Tubercle bacilli, sputum and/or gastric washings. *Negative* to adequate examination.

Available information regarding type of tuberculin, exact dosage, date of test, and precise description of reaction was inadequate so far as pre-military tuberculin tests were concerned. Indeed, in 743 persons, tests had apparently not been made before induction into service. Of the remaining 224 persons, 145 had reacted to earlier tests, 74 had not reacted, and for 5 the results were unknown.

[6] Duplicates of representative roentgenograms, demonstrating the various types of pulmonary lesions under study, are in the project's permanent files at the University of Colorado Medical Center.

Intracutaneous tuberculin sensitivity tests were made *after admission to the army* on every one of the 967 persons in the study group. The results of the tests appear in table 2. Most of these tests were made with purified protein derivative (PPD) but some were made with Old Tuberculin (OT). In addition to reacting to tuberculin, 94 (10 per cent of the whole group) reacted to coccidioidin and 261 (27 per cent) to histoplasmin.[7] Practically all of the coccidioidin and histoplasmin reactors fell in the active group.

3. *Subsequent course of disease.* In this report data covering the subsequent course of tuberculosis, in comparison with its status at the time of first clinical classification by the Army Research Section, apply throughout the period of observation, including both military and post-military periods.

Those patients whose disease was apparently inactive at the time of the first diagnosis by this Research Section and remained inactive throughout the entire period of observation are briefly referred to as the "*inactive-inactive*" group. Actually this category could have been labeled "*inactive*" throughout this report, but use of the term "*inactive-inactive*" emphasizes the factor of time lapse more precisely.

Those with active disease when first studied who subsequently improved comprise the category labeled "*active-improved.*" Those with active disease at first which worsened or relapsed during the follow-up period (or earlier) are referred to as "*active-increased.*" The word "*relapse*" denotes reactivation after arrest has been achieved.

Initial case studies: Initial case studies of 967 military personnel made from January, 1944, to June, 1946, included the following:

1. Thorough personal histories for both earlier civilian and army periods, with full details regarding all possible influential factors. In addition to complete medical histories, case records included amplification of special items such as—

Body build	Living conditions
Climate	Physical exertion
Diet	Pre-military occupations
Duties in the army	Reactions to military duty
Emotional status	Sleep
Exposure to the elements	Social habits
Geographic location	

   Careful attention was directed to possible changes in these and other factors while in the army, compared with pre-military status, and to the elements of living and activity which are peculiar and essential to military duty.
2. Physical examinations with notations of the earlier physical findings reported by previous medical examiners.
3. Review and abstract by the writers of this report of all clinical records and laboratory data related to military illnesses and to all pertinent pre-military illnesses.
4. Review of all known chest roentgenograms, taken at any time. A few such films had been taken ten or more years before entry into the army.

Detailed data covering the initial case studies and the later follow-up interviews were recorded in individual case abstracts. Subsequently these data were tabulated mechanically.

Case studies were initiated on 244 persons in 1944, on 442 in 1945, and on 281 in the first six months of 1946. The follow-up program then extended into September, 1949.

Follow-up interviews: Frequent follow-up interviews, three or more annually for each person, were completed for three or more years after first observation or until death.

[7] The histoplasmin antigen was furnished by Dr. Amos Christie, Department of Pediatrics, Vanderbilt University Hospital; and later by the United States Public Health Service. Coccidioidin antigen was furnished by Dr. Charles Smith of California.

TABLE 3

PATIENTS INCLUDED IN STUDY, CLASSIFIED BY LENGTH OF INTERVAL BETWEEN FIRST AND FINAL INTERVIEWS

Interval Between First and Final Interviews	Patients Included in Study		
	Total	Those Who Died of Tuberculosis	Those Who Died of Other Causes*
Total	967	20	15
Less than one year	7	7	—
One year, less than 2	7	5	2
2 years, less than 3	11	8	3
3 years, less than 4	367	—	8
4 years, less than 5	440	—	—
5 years and more	135	—	2

* Before death from nontuberculous causes 10 of these 15 had relapses.

By means of temporary duty trips throughout the country, the medical staff of the Army Research Section personally interviewed each ex-patient at least twice and up to five or six times during the follow-up period. Each personal follow-up interview included full interval history, both medical and personal, with repeated roentgenograms, physical examinations, and laboratory analyses as indicated. At additional quarterly intervals detailed follow-up examinations were done by mail.

Follow-up observations were completed for a total of 49,468 person-months *after the first interview*, or an average of 51.2 months per person. Excluding the 35 who died during the study, the average follow-up period was 52 months, or 4⅓ years, for each person.

Data in table 3 show that all surviving patients were followed for three years or more. The difference in the interval between first and final interviews hinges on the fact that the project was terminated in September, 1949. Consequently, patients first diagnosed by the Army Research Section in the spring of 1946 could not be observed for much longer than three years. On the other hand, those who were diagnosed tuberculous early in 1944 were followed for five years or more.

From earlier roentgenograms and related medical and personal information of dependable nature, evidence of the time of occurrence of previously present active or inactive tuberculosis was found. In these instances it seemed logical to compute the *over-all* observation period from the first definite diagnosis of tuberculosis or from the first available "positive" chest roentgenogram. On this basis the total observation period was 80,568 person-months, an average of 83.3 months for each, or slightly less than 7 years. This approach seems consistent with the accepted concept of tuberculosis as a disease with fluctuating degrees of activity.

Among follow-up studies of tuberculous patients, this project is unique in that no patient was lost to view because of inability to locate him or because of his failure to cooperate. This unusual feature must be attributed to the close control exerted by the army during the military period, and also to cooperation by the Veterans Administration and numerous other public and private hospitals during the post-military period. According to Sol L. Warren (2) in 19 other follow-up studies of tuberculous ex-patients, the percentage of patients lost over an average follow-up period of 5.2 years has ranged from 2 to 42 per cent.

TABLE 4

PATIENTS WITH SPECIFIED SUBSEQUENT COURSE OF DISEASE, CLASSIFIED BY STATUS OF DISEASE AT FIRST DIAGNOSIS BY THE ARMY RESEARCH SECTION

Status of Disease at First Diagnosis	Patients with Specified Subsequent Course of Disease						
	Total	Inactive-Inactive		Active-Improved		Active-Increased	
		Number	Per Cent of Total	Number	Per Cent of Total	Number	Per Cent of Total
Total	967	267	27.6	245	25.3	455	47.1
Active	625	—	—	215	34.4	410	65.6
Apparently inactive	342	267	78.1	30	8.8	45	13.2

ANALYSIS OF FINDINGS

The group studied consisted of 967 military personnel, of whom 625 (65 per cent) were judged to have active minimal pulmonary tuberculosis at the time of the first clinical classification by the Army Research Section, while the remaining 342 had apparently inactive disease.

At the time of the first interview, 786 patients were hospitalized for tuberculosis and 62 for other causes, while 119 were outpatients. All had been accepted for active military duty following physical examinations which included roentgenograms of the chest in 95 per cent of the cases. This fact implies that all were apparently healthy adults.

The primary purpose of this study was to ascertain what happened under war conditions to military personnel who developed minimal pulmonary tuberculosis while in service. It was also highly desirable to determine the reliability or unreliability of certain criteria for acceptance or rejection of recruits for military service, such as roentgenographic abnormalities and previous history of active tuberculosis. In table 4 the subsequent course of disease is pointed out for patients originally diagnosed by the Army Research Section as having active or inactive minimal disease.

Analysis of these data shows that the group of 625 persons with active disease at first study was eventually augmented by 75 persons in whom the disease had originally been labeled inactive, but which became active during the observation period. The group of persons with inactive disease was correspondingly diminished by the transfer of these 75 persons to the active category.

As noted in table 3, 35 patients died during the observation period. Twenty died of tuberculosis and 15 of other causes. Before death from nontuberculous causes, 10 of these 15 had experienced a relapse of active tuberculosis.

Persons with Active Disease

During the period of observation, two-thirds of the 625 persons with active disease when first diagnosed experienced relapse or became worse and one-third improved.

TABLE 5

PATIENTS WHO HAD PRE-MILITARY HISTORIES OF ACTIVE TUBERCULOSIS AND SUBSEQUENT COURSE OF THE DISEASE, CLASSIFIED BY FORM OF TUBERCULOSIS

Form of Tuberculosis	Total	Patients Who Had Relapses in Service		Patients Who Had Relapses After Discharge		Patients Who Remained Well	
		Number	Per Cent of Total	Number	Per Cent of Total	Number	Per Cent of Total
All forms	94	56	60	12	13	26	28
Pulmonary tuberculosis	43	19	44	6	14	18	42
Pleural effusion	37	27	73	4	11	6	16
Extrathoracic tuberculosis	14	10	71	2	14	2	14

TABLE 6

PATIENTS WHO HAD ACTIVE TUBERCULOSIS OF SPECIFIED TYPE DURING MILITARY SERVICE PRIOR TO WORLD WAR II, CLASSIFIED BY SUBSEQUENT COURSE OF DISEASE

Subsequent Course of Disease	Total	Patients with Active Extrathoracic Disease	Patients with Active Pulmonary Parenchymal Lesions
All courses	18	11	7
Relapses during World War II	11	7	4
Relapses in post-military period	3	2	1
Remained well	4	2	2

Ninety-four of these 625 persons with active disease had histories of pre-military tuberculosis with treatment and arrest. It is indicated in table 5 what happened to these 94 patients during the military and post-military periods.

Although the figures are very small, it is obvious that those who had had pulmonary parenchymal tuberculosis previously fared far better (42 per cent remained well) than those who had had pleural effusion or extrathoracic tuberculosis (16 and 14 per cent, respectively, remained well).

In addition to the 94 persons diagnosed as being actively tuberculous in pre-military life, 18 had developed tuberculosis during a term of military service prior to World War II and had apparently achieved arrest of their disease. Detailed information regarding these 18 patients is shown in table 6.

Altogether 112 persons had been diagnosed and treated for active tuberculosis at some time before World War II. In this group of 112 persons, the time interval between previous illness and relapse varied widely from six months to approximately ten years. For those patients whose previous illnesses had occurred in the pre-military period, the time interval between the previous episode and admission into the army showed little relation to relapse after induction; on the other hand, duration of previous treatment proved to have an important influence upon the probability of later relapse (table 7).

TABLE 7

PATIENTS WITH HISTORIES OF ACTIVE TUBERCULOSIS PRIOR TO WORLD WAR II AND THOSE WHO HAD RELAPSES DURING OBSERVATION PERIOD, CLASSIFIED BY DURATION OF BED REST

Duration of Bed Rest	Total	Patients Who Had Relapses During Observation Period	
		Number	Per Cent of Total
Total	112	82	73
Less than six months	68	66	97
Six months and longer	44	16	36

Comment: Despite "acceptable" roentgenograms, the majority of patients with histories of active tuberculosis before admission to the army in World War II experienced a relapse of their disease during military service. Of 43 persons accepted for military duty despite a previous history of active pulmonary tuberculosis, 19 had relapses during service, 6 after discharge from the army, and 18 remained well. Of 37 accepted for military duty despite a previous history of pleural effusion, 27 had relapses in service, 4 after discharge, and only 6 remained well. Of 14 accepted for military duty despite a previous history of extrathoracic tuberculosis, 10 experienced relapses in service, 2 after discharge; and only 2 remained well (table 5).

Treatment for the earlier episode had varied in duration from two to eighteen months. As may be seen in table 7, of those who had had less than six months of bed rest, nearly three times as many had relapses during the course of this study as did those who had had six months or more of bed rest. Even though the numbers involved are admittedly small, few findings in this report possess greater significance. It is worth repeating that the entry roentgenograms for all but 2 of these 112 persons were within the limits of acceptability for military service. Here is an instance in which the history had greater prognostic significance than the roentgenogram.

Persons with Apparently Inactive Disease

The subsequent course of the disease in 342 patients with apparently inactive minimal pulmonary tuberculosis is given in table 8.

The 52 persons who developed active pulmonary tuberculosis after discharge from the army experienced relapses at intervals, approximately 10 each year, up to four or more years between discharge and illness.

If earlier clinical records and chest roentgenograms had not been reviewed, the 30 cases of pre-military active disease would have been overlooked. In other words, 267 personnel showed evidence of inactive tuberculosis during and following service in the army, but 30 of them had had authenticated active disease prior to induction or enlistment. Of these 30, 7 had had pleural effusion; 3 had had extrapulmonary involvement; and 20, active pulmonary disease; 15 of the last-mentioned group had had sputum or gastric washings positive for tubercle bacilli.

If such adequate and frequent follow-up studies had not been completed, some of the 75 persons in whom active tuberculosis developed after the initial interview would still be rated in the records as having inactive disease. As the

TABLE 8

PERSONS WITH APPARENTLY INACTIVE MINIMAL PULMONARY TUBERCULOSIS AT TIME OF FIRST CLINICAL EXAMINATION BY THE ARMY RESEARCH SECTION, CLASSIFIED BY SUBSEQUENT COURSE OF DISEASE

Subsequent Course of Disease	Persons with Apparently Inactive Minimal Pulmonary Tuberculosis	
	Number	Per Cent Distribution
Total	342	100.0
Remained inactive throughout study	237	69.3
Apparently inactive at first examination	105	30.7
Active in pre-military life; inactive later	30	8.8
Developed active disease in military service and later	75	21.9
Developed active pulmonary tuberculosis after military discharge	52	15.2
Developed active pulmonary tuberculosis during military service*	21	6.1
Developed pleural effusion during military service*	2	0.6

* But af ter first appraisal for this study.

follow-up program was prolonged, more and more instances of "first demonstrable active tuberculosis" have occurred. Seventy-three of the 75 cases of active disease which developed after the first appraisal were in the form of active pulmonary disease, confirmed by the presence of tubercle bacilli in the sputum or gastric washings in 65, or almost 90 per cent; the other 2 were pleural effusions, one confirmed by demonstration of tubercle bacilli in the pleural fluid, and the other by subsequent appearance of active pulmonary disease.

In summary, if patients are grouped by status of disease at the time of first clinical classification, the number with apparently inactive disease was 342. Yet 105 of these 342 did have active tuberculosis at some other time, either preceding or following the original interview.

Entry Roentgenograms

Chest roentgenograms which had been taken at entry into the army were procured and reviewed for 918 (95 per cent) of the 967 military personnel under study. Such roentgenograms had not been taken for the remaining 49 persons prior to their military service.

The results of examination of these 918 roentgenograms are shown in table 9. The interpretations enumerated were made retrospectively by physicians of the Army Research Section and are not those of the original examiners. It is important to note that in 103 cases the lesions were thought by the Army Research Section to be of exudative character.

In 279 instances "scalloped" densities were noted at the apex or apices of the lungs without immediately subjacent parenchymal scars. The persons with these shadows (presumably pleural "caps" or fat pads) did no better and no worse

TABLE 9

ROENTGENOGRAMS* TAKEN UPON ENTRY INTO THE ARMY, CLASSIFIED BY RETROSPECTIVE INTERPRETATION OF PHYSICIANS IN THE ARMY RESEARCH SECTION

Retrospective Interpretation	Roentgenograms* Taken upon Entry into the Army	
	Number	Per Cent Distribution
Total	918	100.0
Normal†	271	29.5
Parenchymal changes of fibrotic, fibronodular, or fibrocalcific nature	542	59.0
Exudative lesions	103	11.2
With hilar and primary-complex calcifications	70	7.6
Without calcifications	33	3.6
Pleural effusion	2	0.2

* Prior to service in World War II roentgenograms had not been taken of 49 military personnel who were included in this study.

† "Normal," or showed hilar and "primary-complex" calcifications only.

TABLE 10

PATIENTS WHOSE ENTRY ROENTGENOGRAMS SHOWED APPARENTLY INACTIVE PULMONARY PARENCHYMAL DENSITIES AND SUBSEQUENT COURSE OF DISEASE, CLASSIFIED BY TYPE OF SCAR

Type of Scar	Patients with Specified Subsequent Course of Disease							
	Total	Inactive-Inactive		Active-Improved		Active-Increased		
		Number	Per Cent of Total	Number	Per Cent of Total	Number	Per Cent of Total	
All types	542	248	45.8	118	21.8	176	32.5	
Fibrotic and fibropleural	133	58	43.6	23	17.3	52	39.1	
Fibronodular	267	116	43.4	59	22.1	92	34.5	
Fibronodular-calcific and fibrocalcific	142	74	52.1	36	25.4	32	22.5	

than those without them. In 126 instances one or both of the costophrenic angles were blurred or obliterated, usually with associated deformity of the diaphragm. Persons with these roentgenographic changes did no better and no worse than others without them.

The entry roentgenograms of 542 persons showed apparently inactive pulmonary parenchymal densities. The subsequent course of disease for this group is classified by type of scar in table 10.

The results in table 10 are statistically significant in that the group with calcific elements in the parenchymal scars in their entry roentgenograms did better in every way than would be expected on the basis of chance alone. Among the 142 persons with calcific components, reactivation was less likely and, whenever it occurred, was likely to improve without recurrence.

Analyses of data for persons with parenchymal scars in relation to the presence or absence of additional discrete calcifications outside of these scars failed to show any relation to the course of disease. The occurrence of such discrete calcifications did not follow any pattern consistent with pre-military geographic residence of the officers and soldiers. This statement includes the presence or absence of apparently healed and calcified "primary complexes."

Correlations of entry roentgenograms with various factors of military duty and with subsequent course of tuberculous disease failed to affect the significance quoted throughout this report for other factors under consideration, including age, sex, race, military grade, limited duty compared with general duty, overseas assignment compared with that in continental United States, actual military duties, and military living conditions.

TABLE 11

PATIENTS WHOSE ENTRY ROENTGENOGRAMS SHOWED INACTIVE PULMONARY PARENCHYMAL DENSITIES AND THOSE WHOSE LESIONS EXCEEDED ALLOWABLE* LIMITS, CLASSIFIED BY TYPE OF SCAR

Type of Scar	Total	Patients Whose Lesions Exceeded Allowable* Limits	
		Number	Per Cent of Total
All types	542	190	35.1
Fibrotic and fibropleural	133	30	22.6
Fibronodular	267	92	34.5
Fibronodular-calcific and fibrocalcific	142	68	47.9

* Lesions referred to as exceeding "allowable" limits were between 4 and approximately 7 square centimeters in area. It is not possible to quote here all the references to tuberculosis in Mobilization Regulations No. 1–9 of the War Department, April 19, 1944. These regulations subsequently have been changed. *See* Army Regulations No. 40–115, Department of the Army, Washington 25, D. C., 20 August 1948.

TABLE 12

PATIENTS WHOSE ENTRY ROENTGENOGRAMS SHOWED INACTIVE PULMONARY PARENCHYMAL DENSITIES AND SUBSEQUENT COURSE OF DISEASE, CLASSIFIED BY AREA OF LESION

Area of Lesion	Total	Patients with Subsequent Course of Disease					
		Inactive-Inactive		Active-Improved		Active-Increased	
		Number	Per Cent of Total	Number	Per Cent of Total	Number	Per Cent of Total
Total	542	248	45.6	118	21.8	176	32.5
In excess of allowable* limits	190	91	47.9	44	23.2	55	29.0
Not in excess of allowable* limits	352	157	44.6	74	21.0	121	34.4

* Lesions referred to as exceeding allowable limits were between 4 and approximately 7 square centimeters in area.

The proportion of patients with each type of scar whose lesions exceeded allowable[8] limits is given in table 11.

The behavior of the disease did not appear to differ significantly in the two groups, i.e., in those with larger scar areas as compared with those having smaller scar areas. The data in table 12 show the subsequent course of disease in these two groups, classified by area of lesion.

Comment: The relative infrequency of reactivation of lesions with calcific components and their more favorable prognosis when reactivation did occur probably speak for the age of the lesions. Relapse in pulmonary tuberculosis is most frequent in the first three years after diagnosis of activity. Roentgenographic demonstration of calcification of tuberculous pulmonary lesions in adults is infrequently found in less than three years.

It is possible that some of these calcified lesions were not tuberculous in origin but were healed fungal lesions, e.g., histoplasmosis, even though the occurrence of discrete calcifications did not follow any recognizable pattern consistent with premilitary geographic residence. However, it is worth repeating that practically all of the histoplasmin and coccidioidin reactors fell into the *active* and not into the *inactive* group.

Subsequent Course of Disease in Relation to Military Factors

Military grade or rank: Officers included in this study comprised 19 per cent of the entire group; 17 per cent of the total were enlisted personnel in the upper three grades; enlisted personnel in the lower grades made up the remaining 64 per cent.

The proportion of officers with active disease who improved was larger than in the case of the enlisted men. In accordance with the then existing army regulations, however, officers as a rule could remain in military hospitals somewhat longer than enlisted personnel. One realizes also that officers, by and large, presumably have had more advantages than enlisted personnel in the way of education and a higher standard of nutrition, although the effect of these advantages under military conditions is of course problematical.

The subsequent course of the disease in the three groups is outlined in table 13.

Arm or branch of military service: Approximately 40 per cent of the persons studied were in the Ground Force and an equal proportion in the Service Force. Twenty per cent of the personnel were in the Air Force, at that time an integral part of the army. Actual duties rather than service assignment affected the subsequent course of disease most significantly.

Geographic assignment: Officers and enlisted personnel who did not depart from the continental limits of the United States for any length of time during the period of observation numbered 369. The remaining 598 served in overseas theaters (Transatlantic and Pacific) for periods of six months or more. The duration

[8] Lesions referred to as exceeding "allowable" limits were between 4 and approximately 7 square centimeters in area. It is not possible to quote here all of the references to tuberculosis in Mobilization Regulations No. 1–9 of the War Department, April 19, 1944. These regulations subsequently have been changed. *See* Army Regulations No. 40–115, Department of the Army, Washington, 25, D. C., 20 August 1948.

TABLE 13

PATIENTS WITH SPECIFIED SUBSEQUENT COURSE OF DISEASE, CLASSIFIED BY MILITARY GRADE OR RANK

Military Grade or Rank	Patients with Specified Subsequent Course of Disease						
	Total	Inactive-Inactive		Active-Improved		Active-Increased	
		Number	Per Cent of Total	Number	Per Cent of Total	Number	Per Cent of Total
All grades	967	267	27.6	245	25.3	455	47.1
Commissioned officers	187	60	32.1	57	30.5	70	37.4
Enlisted personnel, first three grades	165	38	23.0	47	28.5	80	48.5
Enlisted personnel, lower grades	615	169	27.5	141	22.9	305	49.6

TABLE 14

PATIENTS WITH SPECIFIED SUBSEQUENT COURSE OF DISEASE, CLASSIFIED BY GEOGRAPHIC ASSIGNMENT OF MILITARY DUTY*

Geographic Assignment of Military Duty*	Patients with Specified Subsequent Course of Disease						
	Total	Inactive-Inactive		Active-Improved		Active-Increased	
		Number	Per Cent of Total	Number	Per Cent of Total	Number	Per Cent of Total
Total	967	267	27.6	245	25.3	455	47.1
Transatlantic theaters	328	60	18.3	80	24.4	188	57.3
Pacific theaters	270	67	24.8	69	25.6	134	49.6
Continental United States	369	140	37.9	96	26.0	133	36.0

* Major portion of time.

of overseas service bore little relationship to the development of active disease or to subsequent course of disease among the active cases.

According to table 14, persons with apparently inactive lesions who remained in the United States were less apt to develop active tuberculosis than those serving overseas. Similarly, those with active lesions who remained in the United States were less apt to worsen.

The personnel who remained in the United States included a higher proportion of persons who had been assigned to limited duty because of inactive pulmonary lesions in the roentgenograms, whereas those who were sent overseas included more of the troops approved for combat duty and, therefore, presumably in all respects in better physical condition. No significant difference was observed in any analysis between those who served in the Transatlantic theaters and those assigned to the Pacific theaters.

Military duties: The military duties to which the personnel studied were assigned are shown in table 15.

TABLE 15

PATIENTS WITH SPECIFIED SUBSEQUENT COURSE OF DISEASE, CLASSIFIED BY TYPE OF MILITARY DUTY

Type of Military Duty	Patients with Specified Subsequent Course of Disease						
	Total	Inactive-Inactive		Active-Improved		Active-Increased	
		Number	Per Cent of Total	Number	Per Cent of Total	Number	Per Cent of Total
All types	967	267	27.6	245	25.3	455	47.1
Outdoors	650	170	26.2	167	25.7	313	48.2
With combat duty	313	65	20.8	82	26.2	166	53.0
Without combat duty	113	26	23.0	36	31.9	51	45.1
Light or moderate work without combat duty	224	79	35.3	49	21.9	96	42.9
Indoors	317	97	30.6	78	24.6	142	44.8
Heavy work	71	21	29.6	20	28.2	30	42.3
Light to moderate work	246	76	30.9	58	23.6	112	45.5
Limited duty	172	95	55.2	28	16.3	49	28.5
General duty	795	172	21.6	217	27.3	406	51.1

In general, personnel assigned to outdoor duties did almost as well as those assigned indoors. When the group with outdoor duty is broken down into its component parts, those engaged in combat fared worse, as might be expected, while those assigned to light or moderate work outside (without combat duty) fared the best. In most respects, however, the differences noted between the outdoor and the indoor groups are not great.

Comment: These findings substantiate the general medical opinion that heavier physical exertion plus exposure to the elements and the increased emotional strain which are inherent in heavier duty, and particularly in combat, increase the likelihood of worsening in active disease. With outdoor duty the relative number of those who remained well increased significantly with a decrease in the heaviness of their duties; conversely, the relative number of those who experienced relapse with active disease diminished with decreasing heaviness of duty.

Physical exertion, exposure to the elements, and increase of stress and strain obviously are inherent in combat and in prisoner-of-war status. Analyses of these individual factors did not alter the relations just detailed between actual duties and course of disease.

Limited duty: Among the "limited duty" troops relatively more persons with inactive lesions remained well than developed active tuberculosis. This finding is explained in part by the proportion of persons with "inactive" chest roentgenograms among those classified for "limited duty." However, persons with active tuberculosis among the "limited duty" troops did no better than those who had been on general duty So far as the risk of tuberculosis was concerned,

"limited duty" in continental United States was the safest place for the "poor risk" (*see* table 15).

The significance of "limited duty" varied somewhat in the different periods throughout World War II according to the needs for manpower, as reflected in changes of regulations regarding "limited duty." Furthermore, the actual limitations of exertion ("protection") afforded by "limited duty" varied widely according to the needs of the local commander. In general, this classification was designed to place the officer or soldier in an assignment which involved less strenuous duties and more regular and shorter hours of duty. Upon detailed questioning, practically all of the 172 persons who had been on "limited duty" stated that their assignments fitted the foregoing standards.

Duration of military service at time of first clinical classification: A significant correlation was found between the duration of military service and first diagnosis. Of 625 persons with active minimal pulmonary tuberculosis at first diagnosis, *31 developed active disease within less than six months of service, 82 (13 per cent) developed active disease within the first year of service, and 224 (36 per cent) by the end of their second year of service.*

The duration of military service at the time of the first diagnosis for both active and inactive cases is given in detail in table 16.

Comment: The fact that 82 persons of military age developed active tuberculosis within less than twelve months of military service suggests that many were infected before entry into the army. If it had been feasible to do tuberculin and other skin tests on all recruits, including these, routinely before entry it would now be possible to say how many of the 82 were negative at that time, and light might be thrown on the rapidity of infection and the development of active clinical tuberculosis in military personnel after infection. It is remotely possible that some of the 82 were in-

TABLE 16

CASES DIAGNOSED ACTIVE AND APPARENTLY INACTIVE AT TIME OF FIRST DIAGNOSIS BY THE ARMY RESEARCH SECTION, CLASSIFIED BY LENGTH OF MILITARY SERVICE AT THAT TIME

Length of Military Service Prior to First Diagnosis	All Cases		Active Cases		Inactive Cases	
	Number	Per Cent Distribution	Number	Per Cent Distribution	Number	Per Cent Distribution
Total	967	100.0	625	100.0	342	100.0
Less than one year	191	19.8	82	13.1	109	31.9
Less than 3 months	58	6.0	17	2.7	41	12.0
3 months, less than 6	33	3.4	14	2.2	19	5.6
6 months, less than 12	100	10.3	51	8.2	49	14.3
One year, less than 2	222	23.0	142	22.7	80	23.4
2 years, less than 3	255	26.4	177	28.3	78	22.8
3 years, less than 4	170	17.6	137	21.9	33	9.6
4 years, less than 5	67	6.9	46	7.4	21	6.1
5 years, less than 10	33	3.4	25	4.0	8	2.3
10 years and more	29	3.0	16	2.6	13	3.8

TABLE 17

PATIENTS WITH SPECIFIED SUBSEQUENT COURSE OF DISEASE, CLASSIFIED BY STATUS AS PRISONER-OF-WAR

Status as Prisoner-of-War	Patients with Specified Subsequent Course of Disease						
	Total	Inactive-Inactive		Active-Improved		Active-Increased	
		Number	Per Cent of Total	Number	Per Cent of Total	Number	Per Cent of Total
Total	967	267	27.6	245	25.3	455	47.1
Prisoner-of-war	48	4	8.3	15	31.3	29	60.4
Never prisoner-of-war	919	263	28.6	230	25.0	426	46.4

fected just before entry into service but would not have reacted to tuberculin because they were still in the "pre-allergic" state; a few positive reactors could have been recent converters. Tuberculin tests, repeated at appropriate intervals, both before induction and after admission to military service would help to identify[9] the time, place, and source of infection and would add one more opportunity to evaluate the early and remote hazards of recent conversion of the tuberculin test and therefore of recent infection. A negative tuberculin test on discharge would exclude service-connected tuberculosis in the vast majority of circumstances.

Prisoners-of-war: Only 48 of the 967 persons included in this study had been imprisoned by the Germans or the Japanese. Captured American soldiers who had been confined to Japanese prisons remained in prison for longer periods, were subjected to greater dietary restrictions with resultant loss in weight, more exhausting physical labor, and greater emotional strain; for these reasons they did less well than prisoners in the German camps. It is obvious from table 17 that the ex-prisoners fared worse in all respects than did the non-prisoners.

Comment: It must be realized that some of the former prisoners-of-war who developed tuberculosis in captivity died or their disease progressed to advanced stages before return to continental United States; consequently, they would not have been eligible for inclusion in this study of *minimal pulmonary tuberculosis*.

Change in emotional status: An attempt was made to analyze the change in emotional status among the personnel studied when subjected to military conditions. Although considerable data were assembled regarding such emotional changes, it proved difficult to relate them to the subsequent course of disease.

Subsequent Course of Disease in Relation to Non-Military Factors

Age group: So far as increase in active disease was concerned, the younger age groups fared worse than the older. The proportion of those with active disease

[9] This type of epidemiologic information is more likely to become available and valuable in the restricted quarters of a ship and the close contacts of a ship's personnel (*see* reference 9).

TABLE 18

PATIENTS WITH SPECIFIED SUBSEQUENT COURSE OF DISEASE, CLASSIFIED BY AGE GROUP

Age Group	Patients with Specified Subsequent Course of Disease						
	Total	Inactive-Inactive		Active-Improved		Active-Increased	
		Number	Per Cent of Total	Number	Per Cent of Total	Number	Per Cent of Total
All age groups	967	267	27.6	245	25.3	455	47.1
Less than 20 years	30	13	43.3	4	13.3	13	43.3
20-24 years	257	54	21.0	57	22.1	146	56.4
25-29 years	308	80	26.0	83	26.9	145	47.1
30-34 years	206	61	29.6	66	32.0	79	38.3
35-39 years	122	42	34.4	27	22.1	53	43.4
40 years and older	44	17	38.6	8	18.2	19	43.2
Less than 25 years	287	67	23.3	61	21.3	159	55.4
25-34 years	514	141	27.4	149	29.0	224	43.6
35 years and older	166	59	35.5	35	21.1	72	43.4
Median age	28.2	29.2		28.7		27.4	

who showed improvement was highest in the group of persons twenty-five to thirty-four years of age.

It is interesting that Long and Jablon (1) found that the peak of "breakdown" with tuberculosis among men without lesions at induction was at the younger ages of nineteen to twenty-two years, while "breakdowns" among men with lesions at induction occurred at older ages.

In table 18 the subsequent course of disease for persons in the various age groups is outlined. Because of the small numbers involved, findings for the combined age groups possess greater significance.

When median ages are computed, those with inactive disease which remained inactive were the oldest by a slight margin, while persons with active disease which became worse were the youngest.

In the age group twenty to twenty-four, early worsening or later relapse occurred in one of every 2 patients. In each of the other age groups early worsening occurred in approximately one of every 3 patients. The best records were made by persons twenty-five to thirty-four years of age. Correlation of age with sex, race, military grade, actual duties, and other factors did not seem to alter the above observations and their significance.

Mode of transportation to hospital: The mode of transportation to the hospital seemed to bear little relation to the course of disease in the patients with active minimal tuberculosis. Of the entire group studied, 813 patients had traveled to hospitals by ship or train, and 154 had traveled by airplane.

Comment: Attention is directed to the special study (3) on "Air Evacuation of Tuberculous Military Patients" which included persons with *all* stages of pulmonary tuberculosis, but *most of whom had advanced disease.* This report indicated that those

TABLE 19

PATIENTS WITH SPECIFIED SUBSEQUENT COURSE OF DISEASE, CLASSIFIED BY CHANGE OF WEIGHT DURING OBSERVATION PERIOD

Change in Weight During Observation Period	Patients with Specified Subsequent Course of Disease						
	Total	Inactive-Inactive		Active-Improved		Active-Increased	
		Number	Per Cent of Total	Number	Per Cent of Total	Number	Per Cent of Total
Total	967	267	27.6	245	25.3	455	47.1
None or gain*	648	194	29.9	169	26.1	285	44.0
Loss	319	73	22.9	76	23.8	170	53.3

* Five patients reported gains in weight.

brought to the hospital by airplane fared better than did those who traveled by slower and more fatiguing routes.

Change in weight: During the observation period, 319 patients lost weight; 41 of this group had been prisoners-of-war. Only 5 reported gains in weight. The subsequent course of disease for the two principal groups appears in table 19.

Those who had lost weight seemed more prone to develop active disease and to handle active disease less well. It is not possible to determine whether the loss of weight represented "cause or effect." Probably the preceding weight loss in these persons resulted from factors inherent in some types of military duty which might also affect adversely the course of tuberculosis.

Type of onset: The indispensable value of the chest roentgenogram is indicated by the fact that, in 391 of the 625 persons with active tuberculosis at first clinical classification, initial recognition of the presence of pulmonary tuberculosis, despite the absence of suspicious symptoms, was due to routine roentgenograms.

As stated previously, in 594 of the entire group studied, the tuberculous disease was first recognized on roentgenograms, while the remaining 373 persons reported various symptoms, although none was significantly ill.

The type of onset, with or without symptoms, did not bear a significant relationship to the course of disease. One-half of those presenting symptoms proved to have inactive tuberculosis and required only simple treatment for upper respiratory infections.

Physical signs: As shown in table 20, in 737 persons abnormal physical signs over the lungs were completely absent. Persons with persistent rales were more apt to have recurrence of activity than those without rales. Also those with active disease and persistent rales were more apt to get worse than those without rales.

Comment: It will not be forgotten that active minimal pulmonary tuberculosis may be present without abnormal physical signs, such as rales, and indeed without symptoms. Very rarely, active minimal pulmonary tuberculosis, as proved by culturing tubercle bacilli from the gastric washings, may be present not only without symptoms and without physical signs but even without demonstrable roentgeno-

TABLE 20

PATIENTS WITH SPECIFIED SUBSEQUENT COURSE OF DISEASE, CLASSIFIED BY PHYSICAL SIGNS OVER THE LUNGS

Physical Signs over the Lungs	Patients with Specified Subsequent Course of Disease							
	Total	Inactive-Inactive		Active-Improved		Active-Increased		
		Number	Per Cent of Total	Number	Per Cent of Total	Number	Per Cent of Total	
Total	967	267	27.6	245	25.3	455	47.1	
Normal	737	223	30.3	205	27.8	309	41.9	
Rales, with or without other abnormal signs	148	9	6.1	28	18.9	111	75.0	
Other abnormal signs only	82	35	42.7	12	14.6	35	42.7	

graphic changes. On the other hand, rales may persist indefinitely over the upper lobes in persons who have made perfect recoveries from active pulmonary tuberculosis and who are leading and continue without relapse to lead normal lives. In these cases, however, a history of active tuberculosis is usually present, and the roentgenogram will almost invariably show ample evidence of the past conflict.

Laboratory studies: Adequate bacteriologic studies were done on 616 of the 625 persons with active disease, while 9 examinations were inadequate. In 537 cases results were positive for *M. tuberculosis* and in 79, persistently negative. Tubercle bacilli were cultured from the gastric washings or sputum of 3 of the group of 267 persons in whom the disease was judged inactive by all other studies and by subsequent course. In these 3 instances guinea pig inoculations were negative for tubercle bacilli.

Single erythrocyte sedimentation rates did not prove helpful in diagnosis, determination of disease activity, or prognosis. Early repeated high rates followed by lower and normal rates correlated well with improvement of disease, and a series of low rates followed by high rates correlated with reactivation and worsening of disease. Total and differential leukocyte counts were of no value in diagnosis or prognosis.

It is highly significant that a much higher percentage of patients with active disease that worsened was found among persons with positive bacteriologic results than in those whose sputum was persistently negative for tubercle bacilli (67 *vs.* 19 per cent) (table 21).

Comment: In former days, the finding of acid-fast bacilli in a stained smear of sputum was considered to be a reliable sign of active pulmonary tuberculosis. In recent years the appearance of an "atypical" acid-fast organism in sputum or gastric washings in persons with or without physical signs but with unmistakable roentgenographic evidence of pulmonary disease has troubled clinicians. This development has stimulated others to attempt to identify and characterize the qualities of the true *M. tuberculosis*. The term "atypical" here includes morphologic, cultural, and pathogenetic abnormalities. The prognostic significance and public health implica-

TABLE 21

PATIENTS WITH SPECIFIED SUBSEQUENT COURSE OF DISEASE, CLASSIFIED BY TYPE OF LABORATORY TEST

Type of Laboratory Test	Patients with Specified Subsequent Course of Disease							
	Total	Inactive-Inactive		Active-Improved		Active-Increased		
		Number	Per Cent of Total	Number	Per Cent of Total	Number	Per Cent of Total	
Total	967	267	27.6	245	25.3	455	47.1	
Sputum tests for tubercle bacilli:								
Positive	255	1	0.4	64	25.1	190	74.5	
Negative	479	163	34.0	128	26.7	188	39.2	
Inadequate examination	24	2	*	8	*	14	*	
Not examined	209	101	48.3	45	21.5	63	30.1	
Gastric lavage cultures for tubercle bacilli:								
Positive	367	2	0.5	127	34.6	238	64.9	
Negative	451	261	57.9	80	17.7	110	24.4	
Inadequate examination	1	—	—	—	—	1	*	
Not examined	148	4	2.7	38	25.7	106	71.6	
Sputum test and gastric lavage examinations for tubercle bacilli:								
Positive by either test	552	3	0.5	178	32.2	371	67.2	
Negative (no positives)	406	264	65.0	65	16.0	77	19.0	
Tests unsatisfactory	9	—	—	2	*	7	*	

* Percentage not shown with so small a base.

tions of the presence or absence of tubercle bacilli in the sputum have always been important but have acquired new significance in this "era" of chemotherapy. Although in these lines the emphasis seems to be placed on the finding of tubercle bacilli, the diagnosis of active tuberculosis can and should be made in certain well-known instances in the absence of demonstrated *M. tuberculosis*.

Since acute tuberculous pleurisy with effusion is, after all, a form of pulmonary tuberculosis, often unwisely considered benign, and occasionally associated with demonstrable *minimal* pulmonary parenchymal disease, pertinent comparison might here be made with the recently reported study (4) of pleural effusion in military personnel. This study was carried out concurrently with the present report of minimal pulmonary tuberculosis.

Of 42 persons whose original pleural effusions were not examined adequately by culture or animal inoculation, 71.4 per cent later developed active pulmonary tuberculosis; of 48 persons with negative fluids by culture and/or guinea pig test, 60.5 per cent later developed active pulmonary tuberculosis; of 51 persons whose original fluids were positive for tubercle bacilli, 64.7 per cent later developed active tuberculosis. In this instance it was concluded that routine bacteriologic studies of pleural fluid were of doubtful dependability for either etiologic diagnosis or prognosis. Nevertheless, if necessary, repeated efforts should be made to find the tubercle bacillus by culture and by guinea pig inoculation. Examination of large quantities of fluid is apt to be more rewarding than the use of small quantities. Sometimes

TABLE 22

PATIENTS WITH SPECIFIED SUBSEQUENT COURSE OF DISEASE, CLASSIFIED BY DURATION OF HOSPITALIZATION

Duration of Hospitalization	Patients with Specified Subsequent Course of Disease						
	Total	Inactive-Inactive		Active-Improved		Active-Increased	
		Number	Per Cent of Total	Number	Per Cent of Total	Number	Per Cent of Total
Total	967	267	27.6	245	25.3	455	47.1
Less than 6 months	389	121	31.1	43	11.1	225	57.8
6 months, less than 12	378	92	24.3	163	43.1	123	32.5
12 months or more	152	12	7.9	35	23.0	105	69.1
Not hospitalized	48	42	87.5	4	8.3	2	4.2

guinea pig inoculation is positive when cultures are negative. Under any circumstances, a "positive" guinea pig is very convincing.

Duration of hospitalization: This study began early in 1944 and the last new patient was added in June, 1946, preceding the era of chemotherapy for tuberculosis. Streptomycin with or without PAS was given later to a number of patients who experienced relapses in the latter part of the follow-up period of observation. The subsequent course of disease in relation to months of hospitalization for the military bout of minimal disease is shown in table 22.

From this table, it appears that of 389 persons hospitalized less than six months, the disease worsened in 57.9 per cent; of 378 hospitalized six through eleven months it worsened in 33 per cent; but, of 152 hospitalized twelve or more months, the disease worsened in 69 per cent. This seemingly strange inconsistency is due to the fact that 90 persons in this last group of 152 had prolonged hospitalization because of early worsening or prompt progression of disease. The worsening of the disease in these 90 patients consisted of progressive pulmonary disease or extrathoracic spread of disease, or both, within four to six months after the first clinical diagnosis by the Army Research Section. If these 90 are excluded, the revised figures would read: Of 62 patients hospitalized twelve months or more, the disease became worse in 29 per cent. It is also important to note that 11 of these 90 persons had had previous diagnosis and treatment for tuberculosis. In 6 of these 11, the former episode had been pleurisy with effusion; in one, tuberculous cervical lymphadenopathy; and in 4, pulmonary parenchymal disease. Early worsening in the group of 90 was not significantly correlated with age and race.

Six persons with active disease were not hospitalized while in the army. Each of these cases represents the results of an understandable difference of opinion concerning interpretation of chest roentgenograms taken upon separation from the army. Four of these patients improved slowly after separation from the service but in two the disease worsened. In all 6 instances, bacteriologic proof of

tuberculosis was obtained during the post-military hospitalization which promptly followed separation from the army.

Comment: The long hospitalization of 225 persons with inactive disease is perhaps an indication that it often takes prolonged observation to be reasonably certain that lesions discovered by roentgenogram do not represent active tuberculous disease. If it does not seem permissible to exclude the 90 with progressive disease from over-all calculations, then it appears that the longer patients with minimal tuberculosis are hospitalized the less well they do, a conclusion in general at variance with experience.

It may be concluded that the patients with active disease hospitalized more than six months fared much better than those treated less than six months. The significance of this observation is not altered when duration of hospital stay is related to age, sex, race, type of onset, entry roentgenogram, weight change before onset, actual duties, or physical signs.

Relapse

Relapses occurred in 485 persons in the later military period or much more often in the post-military period. Active disease had been found in 410 of this group at the time of the first clinical classification, while in 75 the disease was inactive at the time of the first study. The relapses included practically all forms of intrathoracic and extrathoracic tuberculosis. Bacteriologic or pathologic proof of tuberculosis was obtained in 446 (92 per cent) of these relapses.

It is indicated in table 23 that 96 per cent of the observed relapses occurred within three years after the end of treatment for the military episode.

Of the 20 patients who died of tuberculosis, early worsening had occurred in 18 instances; 19 evinced extrathoracic involvement at some time, usually within less than a year after onset of the original illness. Ten of the 15 patients who died of nontuberculous causes also had had relapses of their tuberculous disease. Seven of the deaths from tuberculosis occurred within the first year after onset of the illness during World War II, 5 in the second year, and 8 in the third year.

Surgical therapy was required for 176 patients who had a total of 234 surgical procedures, as shown in the following summary:

Therapeutic pneumoperitoneum	120
Phrenemphraxis	57

TABLE 23

PATIENTS WHO EXPERIENCED RELAPSES, CLASSIFIED BY PERIOD OF TIME AFTER END OF TREATMENT FOR MILITARY EPISODE

Period of Time After End of Treatment for Military Episode	Patients Who Experienced Relapses	
	Number	Per Cent Distribution
Total	485	100.0
Less than one year	98	20.2
One year, less than 2	152	31.3
2 years, less than 3	215	44.3
3 years or more	20	4.1

Pneumothorax	26
Thoracoplasty	26
Lobectomy	5

Relapse in relation to season of year: Of the 730 persons with active minimal pulmonary tuberculosis at some time during this study, the first diagnosis of disease activity was made in 424 in the winter and spring and in 306 during the summer and fall. Persons with "symptomatic onset" of active pulmonary tuberculosis numbered 310; of this group the first diagnosis of activity was made in 218 in the winter and spring and in 92 in the summer and fall.

The findings for relapse in relation to season of the year are more reliable, since these persons were already under frequent periodic observation. Relapse denotes reactivation after achievement of arrest. In 317 (65 per cent) of the 485 patients who had relapses, the relapses occurred (or were recognized) in the winter and spring and in the remaining 168 in the summer and fall.

Comment: Many students of tuberculosis of fifty years ago were inclined to think that initial onset of active pulmonary tuberculosis, worsening of manifest disease, and pulmonary hemorrhage were all influenced by the season of the year. For example, C. L. Minor (5) says "The season of the year has a distinct influence. In the writer's experience the spring months show a preponderance of hemorrhages, and as this is the time when a tubercular patient is best nourished, it may possibly be explained on the grounds of plethora."

More recently Brice R. Clarke (6), in a brief discussion of the seasonal variations in mortality from respiratory tuberculosis, noted that mortality rates in England and Scotland are higher during the winter months and lowest in summer. "Seasonal tuberculosis mortality in England and Wales shows a constant peak for pulmonary tuberculosis in the March quarter. . . . Comparison of the curves of phthisis and of nontuberculous respiratory mortality suggests that the higher winter mortality from phthisis is due to such complications as pneumonia and bronchitis."

Lumiere (7), in an analysis of the mortality tables of Paris during the period 1925–1944, found marked "seasonal variations in non-tuberculous respiratory diseases and definite but less marked variations in phthisis." Mitchell (8), in a study of progression or "spread" of pulmonary tuberculosis during sanatorium residence, noted that the highest incidence of "spread" occurred in the winter and the lowest incidence in the summer and fall. However, he added that these seasonal differences were not statistically significant.

Since the onset of active pulmonary tuberculosis is often asymptomatic and since active disease may come and go without ever having been recognized or recognized only after repeated episodes, and since the diagnosis is often made only by an incidental roentgenogram of the chest, a statistical study of "seasonal" onset is apt to be unreliable. Nevertheless, an effort was made to throw some light, however feeble, upon the question of the most hazardous season of the year so far as onset of active pulmonary tuberculosis and relapse were concerned. Relapse in the present "chemotherapeutic era" remains as yet an unwritten chapter.

Status of Patients at End of Observation Period

In table 24 is indicated the health status in September, 1949, the end of the follow-up period, of patients initially diagnosed as having active and inactive disease.

TABLE 24

PATIENTS DIAGNOSED ACTIVE AND APPARENTLY INACTIVE AT TIME OF FIRST CLINICAL EXAMINATION BY ARMY RESEARCH SECTION, CLASSIFIED BY HEALTH STATUS IN SEPTEMBER, 1949

Health Status in September, 1949	All Patients		Active Patients		Apparently Inactive Patients	
	Number	Per Cent Distribution	Number	Per Cent Distribution	Number	Per Cent Distribution
Total	967	100.0	625	100.0	342	100.0
Alive and well	632	65.4	370	59.2	262	76.6
Showing evidence of active disease	300	31.0	228	36.5	72	21.1
Not ill, but with roentgenographic or other evidence	216	22.3	*	*	*	*
Hospitalized	47	4.9	*	*	*	*
Sick at home	37	3.8	*	*	*	*
Dead	35	3.6	27	4.3	8	2.3
Of tuberculosis	20	2.1	16	2.6	4	1.2
Of other causes	15	1.6	11	1.6	4	1.2

* Data not available.

Nearly two-thirds of all patients included in this program were alive and well in September, 1949, the end of the observation period. Of the patients originally diagnosed active by the Army Research Section, 59 per cent were well in September, 1949, compared with 77 per cent of those whose initial diagnoses by the Army Research Section were apparently inactive. The number of deaths was very small in both groups.

DISCUSSION

One of the most important problems that arose in this study of minimal tuberculosis in military personnel concerned the evaluation of calcified hilar and tracheobronchial lesions and other minimal parenchymal abnormalities seen in roentgenograms of the chest. Were these lesions residuals of past primary or reinfection tuberculosis or were they due to some other infectious process? If due to another infectious process, what was it and what was its prognosis? If these lesions were due to tuberculosis, were they obsolete, "last year's bird nests," "shadows of past conflicts and not present battles?" Were they beyond reactivation or were they still capable of reactivation?

The antecedent, as well as the subsequent, histories of those with apparently inactive tuberculosis are obviously important. Just how sick were those persons originally? What are the criteria of disease activity? How reliable is the estimate of inactivity? What is the probability of relapse during subsequent military service or after discharge? What criteria could be used to determine as quickly as possible activity or potential reactivation?

Immediately available criteria included: A history of household exposure to tuberculosis, a past history of pleurisy with effusion or active pulmonary tuber-

culosis or suspicious symptoms thereof, age of the patient, the number, location, distribution, size and character of the lesions seen in the roentgenogram, and their stability or instability. To these could be added, today, a history of residence in endemic areas for histoplasmosis and/or coccidioidomycosis, and the results of tuberculin, histoplasmin, coccidioidin, and blastomycin skin testing.

The observations on a small number of persons with pre-military histories of active tuberculosis (56 of 94 had relapses in service) indicate how dangerous it is to subject such persons even to the noncombat hazards of military service. Under any circumstances, the chances of developing clinically active tuberculosis in the first few years after infection (9, 10) are by no means insignificant in infancy or negligible under certain circumstances in adult life.[10] The observations here recorded confirm previous experience that the risk of relapse is greatest in the first three to five years after arrest of active disease.

In the last ten years the largest proportion of deaths from tuberculosis has shifted from the young adult group to those who are past forty-five years. In other words, today the largest number of deaths occurs in men who are forty-five and older. This higher proportion of mortality in males at the older ages may be due to reactivation of lesions acquired in early adult life and at a time when tuberculosis was much more prevalent than it is today (11).

So far as the worsening of active disease is concerned, results of this study indicate that patients in the younger age groups fared worse than those in older categories. The proportion of active lesions which improved was highest among those persons of twenty-five to thirty-four years of age. Also, worsening of disease occurred most frequently in the age group twenty to twenty-four. In each of the other age groups, worsening occurred at lower rates, the lowest figure being in the fourth decade.

At the time Mobilization Regulations were set up and this study was started, the relationship of intrathoracic calcifications to coccidioidomycosis and histoplasmosis and the high prevalence of infection with the latter fungus in the Mississippi, Ohio, and Missouri River valleys were not fully appreciated. In any case, the all-important problem was whether the calcified lesions represented healed, active, or potentially active tuberculosis. A negative tuberculin test and coincident positive histoplasmin or coccidioidin test at the time of examination for admission to the army would have excluded the presence of tuberculosis and simplified the disposition of many recruits with these suspicious intrathoracic calcifications.

In the study group of 967 persons, pre-military tuberculin tests had been made on only 224 (25 per cent). Available information regarding the type of tuberculin and dosage was inadequate. Under the stress of mobilization it was not feasible to do tuberculin, histoplasmin, and coccidioidin tests routinely before admission to the army. The exact significance of the observation that practically all of the coccidioidin and histoplasmin reactors fell into the group with

[10] For divergent views, consult: Canetti, G.: Exogenous reinfection and pulmonary tuberculosis: A study of the pathology, Thorax, 1950, *31*, 224; and Ustvedt, H. J.: The relationship between primary and adult tuberculosis, Brit. J. Tuberc., 1946, *40*, 85.

active tuberculosis and not in the group with inactive disease is not perfectly clear but implies that *Histoplasma capsulatum* was not an important agent in the latter group. *The importance of a tuberculin test on entry to the armed forces and again on separation cannot be overemphasized.*

Multiple parenchymal discrete calcified lesions, uniformly "bird-shot" or "buck-shot" in size and density, scattered symmetrically throughout both lungs but more frequent in the lower lobes, a roentgenographic pattern to which early writers gave the name "wheatena,"[11] form a pattern of calcific lesions in persons often without histories of serious pulmonary disease, highly suggestive of histoplasmosis and not tuberculosis (12). In this study, no roentgenograms conformed to this pattern. Otherwise the intrathoracic (especially hilar and tracheobronchial) calcifications of healed lesions of the two diseases, tuberculosis and histoplasmosis, are indistinguishable. "Mulberry" and "halo" calcific lesions are not reliably diagnostic of histoplasmosis.

According to Medlar (11, 13), the fact that today calcified primary lesions are seen more commonly among older than younger adults may reflect the diminishing opportunity during recent decades to become infected with a large dose of tubercle bacilli. Both experimentally and clinically a correlation seems to exist between degree of exposure to infection and therefore, presumably, to the dose of bacilli and the extent of calcification of the primary complex. Although the extent of caseation and consequent calcification are not entirely dependent upon the dose of bacilli, the occurrence of calcification and its extent appear to be closely related to the intensity of the infection.

One of the most important conclusions of the Prophit Survey (14), "Tuberculosis in Young Adults," is that under certain conditions subsequent tuberculosis morbidity is higher in persons not reacting to tuberculin when first observed than in those reacting to tuberculin. This Prophit Survey recorded 210 cases of active tuberculosis developing among 10,000 persons whose roentgenograms were clear on "entry." The morbidity rate was 7.3 per 1,000 per year in persons tuberculin positive on entry and 23.1 per 1,000 in persons negative on entry. To quote from this Survey, "These findings are in agreement with the combined results of twenty-seven other surveys." In other words the chances of developing active tuberculosis were three times as great among observed recent converters of the tuberculin test as among those who had become infected at some indefinite unknown previous period.

J. A. Myers (15) found in a significantly large group of children observed over a period of many years that the child who reacts definitely to the tuberculin test is much more liable to develop clinical tuberculosis during the teen-age period than the child who does not react to the test. In another group of children very carefully studied, however, it was found that those with roentgenographic evidence of calcium deposits in the lung parenchyma, hilum, or both, are not necessarily more liable to fall ill from the clinical disease during the teen-age period than children who react to the tuberculin test and have no roentgenographic manifestations at the time.

[11] So called because it was first seen or described in persons from the United States "wheat belt."

The prognostic significance of a positive tuberculin test is well illustrated in the United States Public Health Service studies of Shirley Ferebee and Carroll Palmer who found that in school children in Puerto Rico and in Georgia, three-fourths and four-fifths, respectively, of clinical tuberculosis developed subsequently among the "positive reactors."

Palmer and Ferebee (16) in their three-year study of 69,000 United States Navy recruits after induction found only 10 per cent to be reactors, but the subsequent morbidity rate per 100,000 person-years was 20 for the nonreactors and 132, or more than six times as much, for the reactors.

Mitchell (17) says:

> In line with previous observations, disease characterized by string-like shadows after six to eight months of rest treatment was less apt to be followed by relapse than was disease characterized by nodular or mixed shadows, although this is not significantly demonstrated in this relatively small sample.

It seems obvious that a nodular lesion is more apt to be caseonecrotic, perhaps even a small filled-in cavity, and therefore more liable to reactivation than a predominantly fibrotic lesion with less caseation and more fibrosis. Estimation of roentgenographic stability or instability suffers somewhat from irregularities of technique and inexperience and difficulty in interpretation. Over a twelve-month period, absence of roentgenographic change "for better or for worse" in the appearance of well-calcified lesions is acceptable evidence of stability.

A familial history of tuberculosis is intriguing for two reasons: first, because of the debatable possibility of inherited susceptibility; and, second, because of the implication of intimate household exposure. The terms "familial history" and "household exposure" are not equivalents. One may have a bad family history of tuberculosis and yet never have been exposed under the same roof to a tuberculous member of the family. Exposure to tuberculosis in the household could mean exposure to a nursemaid or other domestic or to a visiting stranger and not to a member of the family.

If one considers only tuberculin reactors, the prevalence of manifest calcified intrathoracic lesions is highest in those who have been most exposed to infection and reinfection with the tubercle bacillus. Furthermore, much investigation indicates that active pulmonary tuberculosis develops more frequently in persons with visible primary lesions than in persons in whom no such lesions are visible. For example, the Prophit Survey (14) showed that 20 of 45 persons with visible primary lesions developed active tuberculosis, compared with only 28 of 545 persons without visible primary lesions. This observation also suggests the importance of search for the early primary lesion promptly after conversion of the tuberculin test.

The Prophit Survey records a high prevalence of pulmonary tuberculosis in those in household contact with a patient with tuberculosis. Minimal lesions were more frequently progressive in contact groups than in others. The incidence of active disease in contacts was greater when the index case was known to be a patient with tubercle bacilli in the sputum. In the section on "Morbidity Re-

lated to Exposure and Environment," the authors of the Prophit Survey conclude:

> The facts reported in this section, similar to those recorded by many other surveys, underline the extreme importance of the repeated roentgenographic examination of young adults who are exposed to tuberculosis in their homes.

Epidemiologic observations by Madsen, Holm, and Jensen (18) on tuberculosis in Denmark indicated that persons who acquire their infection in the so-called tuberculous milieu are probably subject to more intensive and prolonged exposure to infection with tubercle bacilli than those exposed accidentally. As this difference conceivably might play a role in the course of developing disease, these investigators correlated the frequency of roentgenographic changes in the lungs in "converters" of the tuberculin test with the demonstrated household sources of infection. They concluded that "converters" who acquire their infection in a tuberculous milieu (familial contact, household exposure) have demonstrable roentgenographic changes more than twice as often as have other "converters."

The two surveys of persons exposed to tuberculosis in New York and Philadelphia households made by Beeuwkes and Hahn (19) and Beeuwkes, Hahn, and Putnam (20) demonstrate the risk of exposure to open tuberculosis in the home. The New York survey covered 2,221 persons in 502 families. After certain deductions, 484 cases of manifest disease remained with 1,674 persons exposed to the disease. The risk of manifest disease to those exposed in the household was shown by the high percentages of persons with only positive tuberculin tests, or asymptomatic lesions, or with manifest disease. More than one-half of the infants and young children who began their exposure in infancy and later developed clinical disease did so over a period of twenty years. Among those who were from fifteen to twenty-four years of age at the beginning of exposure, the onset of clinical tuberculosis occurred after a shorter interval. Also a higher percentage of these contacts developed manifest disease.

In brief, manifest disease developed sooner or later among some contacts regardless of the age at which exposure began. Among all the contacts exposed to tuberculosis in these households, 8.3 per cent developed manifest disease. An additional 7.8 per cent of persons with roentgenographic examinations were found to have asymptomatic infections of the adult (apical) type and 0.9 per cent of the childhood type. Of the asymptomatic infection of childhood or adult type observed in 87 persons, 11 (12.6 per cent) progressed to manifest disease, "all indications of their importance as forerunners of clinical disease."

Although the importance of household exposure to tuberculosis is emphasized, many studies show fairly consistently that a very large percentage (44 to 72 per cent) of all tuberculous persons came from families in which neither parent was tuberculous. Of the 967 persons included in this study, 300 (31 per cent) gave histories of exposure to tuberculosis in pre-military life. It has not been possible to correlate reliably with subsequent development of active tuberculosis this often indefinite history of exposure.

Recently Zeidberg and associates (21), in a report on the risk of developing tuberculosis among the children of tuberculous parents, analyzed data collected over a period of twenty-two years in Williamson County, Tennessee, on 733 children of white tuberculous parents with acid-fast bacilli in the sputum and, for comparison, 760 children of white tuberculous parents with clinical and roentgenographic evidence of tuberculosis, including tuberculin allergy, but without acid-fast bacilli in the sputum or "whose sputum was not obtained." The tuberculosis attack rate was 3.3 per 1,000 person-years among children of tuberculous parents with infectious sputum and 1.8 for children of tuberculous parents in whom no tubercle bacilli were demonstrated in the sputum. In the former group the highest rates were in children less than five years of age and in those fifteen to twenty-four years of age. In the noninfectious sputum group, rates were extremely low in persons less than fifteen but rose to a peak at twenty-five to thirty-four years of age. The risk of developing tuberculosis after removal from a tuberculous household was not as great as it was during residence but was nevertheless significant. Among those exposed for the first time at fifteen years of age or later, the interval between infection and disease was short.

Rich (22) says:

> Finally, one cannot neglect the fact that the exposure of children to the opportunity for repeated infection, as is provided by contact with a tuberculous parent, may reasonably be expected to favor the development of a larger number of the more severe latent infections which hold a greater threat of becoming active in later life.

A recent observation by Long and Jablon (1) in their Veterans Administration Medical Monograph on "Tuberculosis in the Army of the United States in World War II" is important. They say that, with the exception of service in the Medical Department, the development of tuberculosis was not significantly related to army arm or occupation. A relatively high rate of development of tuberculosis occurred in the Medical Department, which might have been due to excessive exposure in the occupation itself, either in pre-military life or during military service.

In this discussion, emphasis has been placed upon: (*1*) the great risk of relapse in military service of a person with a previous history of active tuberculosis, whether pulmonary, pleural, or extrathoracic; (*2*) the risk of relapse during the first three years after arrest of active tuberculosis; (*3*) the risk of the recent converter of the tuberculin test and therefore upon the importance of the repeated tuberculin test; (*4*) the importance of the negative tuberculin test in excluding a diagnosis of tuberculosis in persons with calcified lesions in the lungs; (*5*) the importance of household exposure to active tuberculosis; and finally (*6*) the greater risk of developing tuberculosis or relapse of previous infection during young adulthood.

The two most important methods of discovery and control of tuberculosis, the repeated tuberculin test and the repeated roentgenogram of the chest, are indispensable in both civilian life and military service. They should be more readily applicable in the latter. Long and Jablon (1) emphasize the importance of dual

reading of chest roentgenograms at induction stations and note that approximately one-half of the men discharged for tuberculosis had the disease in roentgenographically detectable form at the time of acceptance for service.

* * *

Although time is still lacking for final appraisal of its effect upon remote relapse, chemotherapy has in the last few years altered dramatically the *immediate* prognosis of tuberculosis in all of its forms. Therefore, any recommendations at this time with reference to acceptability or disqualification for admission to the army will surely be subjected to modification in the light of added experience.

From the observations here recorded, however, it is evident that persons with histories of treatment for active pulmonary tuberculosis, or tuberculous pleurisy with effusion, or so-called idiopathic pleurisy with effusion, or extrathoracic tuberculosis (with the possible exception of fistula-in-ano as the sole manifestation of tuberculous disease) should not have been accepted for unrestricted military service. The probability of relapse was minimized if the original disease had been minimal in extent, left well calcified scars, occurred more than five years before induction, and was not accompanied at any time by extrathoracic complications. Although difficult to appraise, limited duty in continental United States gave some measure of protection.

As it is extremely difficult, often impossible, to evaluate quickly and accurately the activity or inactivity of presumably tuberculous intrathoracic lesions (calcific, fibrocalcific, fibronodular-calcific), recourse must be had without fail to tuberculin and other skin tests to assist in the exclusion of tuberculosis.

During military service repeated tuberculin testing of nonreactors and timely roentgenograms of recent converters would assist greatly in the discovery of minimal tuberculosis in its early and most curable phase. The immunization record of every person in the armed forces should carry a record of tuberculin tests. A chest roentgenogram and a tuberculin test for every person at the moment of separation from military service are public health measures of first importance.

Summary

This study of minimal pulmonary tuberculosis as it occurred in the United States Army during World War II was begun in January, 1944; the last patient was included in the project in June, 1946, preceding the era of chemotherapy for tuberculosis. The period of observation was continued until September, 1949. The primary purpose of this study was to ascertain what happened under war conditions to military personnel developing minimal pulmonary tuberculosis while in service. It was also hoped to determine the reliability or unreliability of certain criteria for acceptance or rejection of recruits for military service, such as roentgenographic abnormalities and previous history of active tuberculosis.

After excluding the 35 persons who died during the study, the average follow-up period was fifty-two months for each person, or slightly more than four years. Chest roentgenograms taken on entry into the army were procured and

reviewed for 918 of the 967 military personnel under observation. During the follow-up period, every person in the group was examined three to six times annually for three or more years, and more than twenty-eight films per person were reviewed by physicians of the Army Research Section which conducted the study.

The method of selecting patients for study is believed to have yielded a valid random sample of minimal pulmonary tuberculosis in the army. For example, the median age of all patients included in this study was 28.2 years, compared with 28.8 years for the patients with minimal disease in the army as a whole during the same period.

Of 625 persons with active minimal tuberculosis at the time of the first clinical classification by the Army Research Section, the disease improved in one-third and worsened in two-thirds during the period of observation. Of 342 persons with apparently inactive tuberculosis at first study, 78 per cent remained well, 9 per cent had relapses and later showed improvement, while 13 per cent had relapses with subsequent worsening of their disease.

With respect to increase in active disease, the younger age groups fared worse than the older. The proportion of active cases in which improvement occurred was highest in the group of persons twenty-five to thirty-four years of age.

Eighty-two persons developed active pulmonary tuberculosis within twelve months and 31 of them within six months after entry into military service. Despite "acceptable" roentgenograms, the majority of those with histories of active tuberculosis before admission to the army in World War II had relapses during military service. Of 94 persons with histories of pre-military active tuberculosis, 68 had relapses during service or after discharge. Fourteen of 18 persons with diagnosis and treatment of minimal pulmonary tuberculosis with apparent recovery during earlier military service had relapses after return to duty in World War II. The probability of relapse was greatest in those with histories of pleural effusion (31 of 37), in those with previous extrathoracic tuberculosis (12 of 14), and in those who had less than six months of rest treatment (68 of 112). On the other hand, 30 of these 112 persons (94 plus 18) with histories of active tuberculosis in the pre-military period of World War II remained well while in service and did not experience a relapse subsequently in the period of observation. Unless adequately treated before return to duty, the hazard of relapse during military service after apparent recovery from active tuberculosis is great. The figures involved are admittedly small. These observations were made before chemotherapy became available.

Persons with calcific elements in the parenchymal lesions shown on entry roentgenograms did better in every way than would be expected on the basis of chance alone. In the absence of pre-military tuberculin, histoplasmin, and other skin tests, the implications of this observation are uncertain. In the estimation of activity and potential reactivation, the number, distribution, size, and character of intrathoracic lesions are not as helpful as roentgenographic evidences of stability or instability of the lesions. The value of a negative tuberculin test has been emphasized.

Abnormal physical signs and symptoms were not very helpful in diagnosis.

In 391 (62.5 per cent) of the 625 persons with active tuberculosis at first examination by the Army Research Section, initial recognition of active pulmonary tuberculosis in the absence of suspicious symptoms was due to routine roentgenograms of the chest.

Among the active cases the duration of overseas service bore little relation to "breakdown" with active disease or to the subsequent course of disease. Persons engaged in combat fared the worst, while those assigned to light or moderate work outside without combat duty fared the best.

Assignment of "poor risks" to limited duty within continental United States seemed to afford a measure of protection. Persons with active tuberculosis on limited duty did no better, however, than those on general duty. These findings substantiate the general medical opinion that heavier physical exertion increases the likelihood of "breakdown" with tuberculosis and also increases the likelihood of worsening in active disease.

The type of onset, with or without symptoms, did not bear a significant relationship to the course of disease; one-half of those presenting symptoms proved to have inactive tuberculosis. Persons with persistent rales were more apt to have recurrence of activity than those without rales. Also those with active disease and persistent rales were more apt to get worse than those without rales. Single erythrocyte sedimentation rates did not prove helpful in diagnosis, determination of activity, or in prognosis. "Active" cases with sputum persistently negative for tubercle bacilli did better than "active" cases with sputum positive for tubercle bacilli.

Patients with longer periods of hospitalization did much better than those treated for less than six months. Relapses occurred during military service and more often in the post-military period in 485 persons. Approximately 96 per cent of all observed relapses occurred within the first three years after the end of treatment for the military episode of active disease. Relapses included practically all forms of intra- and extrathoracic tuberculosis.

At the end of the follow-up period in September, 1949, of 625 persons with active minimal tuberculosis at first observation by this Research Section, 370 were well, 228 had active disease, 16 were dead of tuberculosis, and 11 had died of other causes. Of 342 with inactive minimal tuberculosis, 262 were well, 72 had active disease, 4 were dead of tuberculosis, and 4 had died of other causes. Of the 342 rated inactive at first observation, 75 had had relapses and had active disease, 23 while in service and 52 after separation. Of the 20 patients who died from progressive tuberculosis, early worsening had occurred in 18 instances and 19 had extrathoracic spread of disease at some time, usually within twelve months after onset of the original disease. Ten of the 15 who died of nontuberculous causes also had had relapses of their tuberculosis.

In brief, of those diagnosed as having active tuberculosis at the time of the first clinical examination by the Army Research Section, 59 per cent were alive and well at the end of the period of observation, compared with 77 per cent of those diagnosed as having apparently inactive disease.

Sumario

Tuberculosis Pulmonar Mínima

Este estudio de la tuberculosis pulmonar mínima, tal como se observó en el Ejército de los Estados Unidos durante la II Guerra Mundial, fué iniciado en enero de 1944; el último enfermo quedó comprendido en el proyecto en junio de 1946, antes de la era de la quimioterapia en la tuberculosis. El período de observación continuó hasta septiembre de 1949. El propósito primordial del estudio era averiguar qué acontecía en condiciones de guerra al personal militar que manifestaba tuberculosis pulmonar mínima mientras servía en las filas. Se esperaba además determinar la infalibilidad o falibilidad de ciertas pautas empleadas para la aceptación o el rechazo de reclutas para servicio militar, tales como anomalías radiográficas e historia de tuberculosis activa.

Excluídas las 35 personas que fallecieron durante el estudio, el período medio de observación subsecuente fué de cincuenta y dos meses para cada persona, o sea un poco más de cuatro años. Se procuraron y repasaron las radiografías tomadas al ingreso en el Ejército para 918 de los 967 militares mantenidos en observación. Durante el período de observación, cada persona del grupo fué examinada de tres a seis veces anualmente durante tres o cuatro años, y más de 28 radiografías por persona fueron repasadas por médicos de la Sección de Investigación del Ejército que llevó a cabo el estudio.

El método usado para seleccionar los enfermos para estudio parece haber rendido una muestra al azar válida de la tuberculosis pulmonar mínima en el Ejército. Por ej., la edad media de todos los enfermos comprendidos en este estudio fué de 28.2 años, comparado con 28.8 años para todos los sujetos que tuvieron enfermedad mínima en el Ejército en conjunto durante el mismo período de tiempo.

De 625 personas con tuberculosis pulmonar mínima al hacer la primera clasificación clínica la Sección de Investigación del Ejército, la dolencia mejoró en la tercera parte y empeoró en dos terceras partes durante el período de observación. De 342 personas que tenían tuberculosis aparentemente inactiva en el primer estudio, 78 por ciento permanecieron bien, 9 por ciento tuvieron recidivas y después mostraron mejoría, mientras que 13 por ciento tuvieron recidivas con agravación subsigniente de su enfermedad.

Con respecto a aumento de la enfermedad activa, los grupos de menor edad lo pasaron peor que los de mayor edad. La proporción de casos activos en que hubo mejoría alcanzó su máximo en el grupo de personas de veinticinco a treinta y cuatro años de ead.

Ochenta y dos personas manifestaron tuberculosis pulmonar activa en término de doce meses y treinta y una de ellas en término de seis meses de su ingreso en el servicio militar. A pesar de radiografías "aceptables," la mayoría de las que tenían historias de tuberculosis activa antes de su ingreso en el Ejército en la II Guerra Mundial experimentó recidivas durante el servicio militar. De 94 personas con historias de tuberculosis activa premilitar, 66 tuvieron recidivas durante el servicio o después del alta. Catorce de 18 personas con diagnóstico y tratamiento de tuberculosis pulmonar mínima y con curación aparente durante el servicio militar anterior tuvieron recidivas al volver a las filas en la II Guerra Mundial. Las probabilidades de recidivas alcanzaron su máximo en los que tenían historia de derrame pleural (31 de 37), en los que habían tenido anteriormente tuberculosis extratorácica (12 de 14) y en los que habían tenido menos de seis meses de reposo terapéutico (68 de 112). Por otro lado, 30 de estas 112 personas (94 más 18) con historia de tuberculosis activa en el período premilitar de la II Guerra Mundial permanecieron bien mientras servían y no experimentaron recidivas después en el período de observación. A menos que haya tratamiento adecuado antes de volver a las filas, es grande el riesgo de recidivas durante el servicio militar después de la aparente curación de la tuberculosis activa. Las cifras disponibles son reconocidamente pequeñas. Estas observaciones se hicieron antes de contarse con la quimioterapia.

Las personas con elementos calcíficos en las lesiones parenquimatosas reveladas en las radiografías de ingreso lo pasaron mejor en todos sentidos que lo que sería de esperar

a base del azar solamente. A falta de pruebas premilitares con tuberculina, histoplasmina y otras cutirreacciones, las connotaciones de esta observación son inciertas. En la apreciación de la actividad y la reactivación potencial, el número, distribución, tamaño y naturaleza de las lesiones intratorácicas no son tan útiles como los signos radiográficos de estabilidad o inestabilidad de las lesiones. Se recalca el valor de una prueba tuberculínica negativa.

Los signos físicos anómalos y los síntomas no fueron muy útiles en el diagnóstico. En 391 (62.5 por ciento) de las 625 personas con tuberculosis activa en el primer examen por la Sección de Investigación del Ejército, al reconocimiento inicial de tuberculosis pulmonar activa a falta de síntomas sospechosos se debió a las radiografías corrientes del tórax.

Entre los casos activos, la duración del servicio en ultramar guardó poca relación con el "desquiciamiento" con enfermedad activa o con la evolución subsiguiente de la dolencia. Las personas que entraron en combate fueron las que lo pasaron peor, mientras que en las dedicadas a tareas leves o moderadas sin misión de combate fueron las que lo pasaron mejor.

La asignación de los "riesgos malos" a servicio limitado en la porción continental de los Estados Unidos pareció ofrecer cierta medida de protección. Sin embargo, los que tenían tuberculosis activa dedicados a servicio limitado no lo pasaron mejor que los dedicados a servicio general. Estos hallazgos comprueban la opinión médica general de que el ejercicio físico intenso acrecienta las probabilidades de "agotamiento" con tuberculosis y también las probabilidades de agravación en la enfermedad activa.

La forma de iniciación, con o sin síntomas, no guardó relación importante con la evolución de la dolencia; la mitad de los que presentaban síntomas resultaron tener tuberculosis inactiva. Los que tenían estertores persistentes se mostraron más susceptibles que los que no los tenían de experimentar recurrencia de la actividad. Los que tenían enfermedad activa y estertores persistentes fueron también más susceptibles que los que no los tenían de experimentar agravación. Los índices aislados de eritrosedimentación no resultaron útiles en el diagnóstico, la determinación de la actividad o el pronóstico. Los casos "activos" con esputo persistentemente negativo para bacilos tuberculosos lo pasaron mejor que los "activos" de esputo positivo.

Los enfermos que gozaron de períodos más largos de hospitalización lo pasaron mucho mejor que los tratados menos de seis meses. Hubo recidivas durante el servicio militar y más a menudo en el período postmilitar en 425 personas. Aproximadamente 96 por ciento de todas las recidivas observados occurrieron en término de los primeros tres años después de terminar el tratamiento del episodio militar de enfermedad activa. Las recidivas comprendieron prácticamente todas las formas de tuberculosis intra- y extratorácica.

Al final del período de observación subsecuente en septiembre de 1949, de 625 personas con tuberculosis mínima activa al observados por primera vez por la Sección de Investigación, 370 se hallaban bien, 228 tenían enfermedad activa, 15 habían fallecido de tuberculosis y 11 habían muerto de otras causas. De 342 que tenían tuberculosis mínima inactiva, 262 se hallaban bien, 72 tenían enfermedad activa, 4 habían fallecido de tuberculosis y 4 habían muerto de otras causes. De los 342 clasificados como inactivos en la primera observación, 75 habían tenido recidivas y tenido enfermedad activa: 23 mientras servían en las filas y 52 después del alta. De los 20 que fallecieron de tuberculosis evolutiva, en 18 había ocurrido empeoramiento temprano y 19 habían mostrado en alguna ocasión difusión extratorácica de la dolencia, por lo general en término de doce meses de la iniciación de la enfermedad primitiva. Diez de los 15 que fallecieron de causas no tuberculosas también habían experimentado recidivas de su tuberculosis.

En resumen, de los diagnosticados como teniendo tuberculosis activa al hacer su primer examen clínico la Sección de Investigación del Ejército, 59 por ciento se hallaban vivos y bien al final del período de observación, comparado con 77 por ciento en los diagnosticados como teniendo enfermedad aparentemente inactiva.

Resume

Tuberculose pulmonaire a minima

Cette étude de la tuberculose pulmonaire a minima, telle qu'elle a été observée dans l'armée des Etats-Unis durant la Deuxième Guerre mondiale, fut entreprise en janvier 1944; le dernier malade fut compris dans l'étude en juin 1946, avant l'ère de la chimiothérapie antituberculeuse. La période d'observation s'est poursuivie jusqu'en septembre 1949. Le but principal de cette étude était d'obtenir des précisions sur ce qui se produisait dans les conditions de guerre chez les soldats atteints de tuberculose *a minima* durant leur service militaire. On espérait également déterminer la valeur ou l'absence de valeur de certains critères d'incorporation ou de rejet à l'égard du service militaire, tels que les anomalies radiologiques et les antécédents de tuberculose en activité.

Après exclusion de 35 sujets morts au cours de l'étude, la moyenne de la période d'observation fut environ de cinquante-deux mois pour chaque sujet, soit un peu plus de 4 ans. Les clichés radiologiques thoraciques effectués au moment de l'incorporation furent obtenus et analysés pour 918 des 967 membres du personnel militaire en observation. Durant la période d'observation, chaque sujet de ce groupe fut examiné 3 à 6 fois par an pendant trois ans ou plus, et plus de 28 clichés par individu furent analysés par des médecins de la Section de Recherches de l'Armée qui dirigeait cette étude.

On considère que la méthode de sélection des malades dans cette étude a permis d'obtenir une représentation valable de la tuberculose pulmonaire *a minima* dans l'armée. Par example, l'âge moyen de la totalité des malades inclus dans l'étude était 28, 2 année, par comparaison avec 28,8 année chez les malades avec tuberculose *a minima* pour l'ensemble des l'armée durant le même période.

Sur un nombre de 625 individus atteints de tuberculose *a minima* en activité au moment de la classification initiale effectuée par le Service de Recherches de l'Armée, il y a eu un tiers des cas améliorée et une aggravation dans les deux autres tiers durant la période d'observation.

Sur un nombre de 342 individus atteints de tuberculose paraissant stabilisée au moment de l'examen initial, l'état de 78% d'entre eux est nemeuré bon; 9% ont eu des rechutes avec amélioration ultérieure et 13% ont eu des rechûtes avec aggravation de la maladie.

A l'égard de l'accroissement de la tuberculose en activité, les groupes d'âge plus jeune se sont comportés beaucoup plus mal que les groupes plus âgés. C'est dans le groupe des sujets âgés de 25 à 34 ans que la proportion des cas évolutifs où l'amélioration s'est manifestée a été la plus élevée.

La tuberculose pulmonaire en activité s'est manifestée dans les douze mois chez 82 individus et chez 31 d'entre eux dans les six mois qui ont suivi l'incorporation dans l'armée. En dépit de radiogrammes "acceptables", la majorité des malades présentant des antécédents de tuberculose en activité avant l'incorporation dans l'armée durant la Deuxième Guerre mondiale, ont eu des rechûtes au cours de leur service militaire. Sur 94 sujets ayant des antécédents de tuberculose en activité avant leur service militaire, 64 ont eu des rechûtes pendant leur séjour dans l'armée ou après leur libération. Sur 18 sujets, pour lesquels le diagnostic et le traitement de la tuberculose pulmonaire *a minima* avaient été faits et dont la tuberculose paraissait stabilisé pendant leur service militaire antérieur, 14 ont eu des rechûtes quand ils ont repris du service pendant la Deuxième Guerre mondiale. La probabilité de la rechûte était plus élevée dans les cas où l'épanchement pleural antérieur figurait dans les antécédents (31 sur 37), dans les cas de tuberculose extra-thoracique antérieurs (12 sur 14), dans les cas où le traitement par le repos avait duré moins de six mois (68 sur 112). D'autre part parmi ces 112 individus (94 plus 18) ayant des antécédenta de tuberculose en activité avant la période militaire de la Deuxième Guerre mondiale, 30 d'entre eux sont demeurés bien portants pendant leur séjour dans l'armée et n'ont pas eu de rechûte durant la période d'observation. A moins d'être traités de façon adéquate avant leur retour dans l'armée, le risque de rechûte pendant le service militaire après

stabilisation d'une tuberculose évolutive, est grand. Les auteurs admettent que les chiffres de cette statistique sont peu élevés. Ces observations ont été faites avant l'introduction de la chimiothérapie.

L'état des sujets, dont les lésions parenchymateuses présentaient des éléments calcifiés visibles sur les clichés effectués à l'incorporation, a été meilleur de toutes les manières qu'on ne pouvait l'espérer, ce qui ne pouvait être attribué au hasard seulement. En l'absence d'épreuve à la tuberculine, à l'histoplasmine et d'autres tests cutanés effectués avant l'incorporation, les implications de cette observation demeurent imprécises. Dans l'évaluation de l'activité et de la réactivation éventuelle, le nombre, la répartition, l'importance et le caractère des lésions intra-thoraciques sont moins utiles que les indices radiologiques de stabilité ou d'instabilité des lésions. La valeur du test négatif à la tuberculine est accentuée.

Les signes physiques anormaux et les symptômes sont relativement peu utiles pour le diagnostic. Chez 391 sujets (62,5%) sur 625 cas de tuberculose en activité au moment du premier examen par la Section de Recherche de l'Armée, le dépistage initial de la tuberculose pulmonaire en activité en l'absence de signes suspects était dû aux radiographies thoraciques systématiques.

Parmi les cas évolutifs il y avait peu de corrélation entre la durée du service outre-mer et l'apparition de la phase évolutive de la tuberculose ou le cours ultérieur de la maladie. L'état des combattants a le plus souffert, tandis que celui des sujets affectés au service auxiliaire a été meilleur.

L'affectation des "mauvais cas" (poor risks) au Service auxiliaire sur le territoire des Etats-Unis paraissait constituer une mesure de protection. Toutefois, l'état des individus porteurs de lésions évolutives qui furent affectés à un service restreint n'a pas été meilleur que celui des sujets affectés au service général. Ces résultats corroborent l'opinion médicale générale que, dans la tuberculose en activité, le surmenage physique augmente les chances d'apparition de la phase évolutive et de l'aggravation de la tuberculose.

Les modalités du début de la tuberculose apparente ou latente étaient dépourvues de corrélation significative avec le cours de la maladie; la tuberculose stabilisée a été démontrée chez la moitié des sujets présentant des symptômes. L'activation des lésions est plus apte à survenir chez les individus avec râles persistants que dans les cas où les râles font défaut. De même, dans les cas de tuberculose en activité avec râles persistants, l'aggravation a été plus apte à se produire que lorsque les râles n'existaient pas. La détermination de la vitesse de sédimentation globulaire seule n'a pas été considérée utile au diagnostic, à l'évaluation de l'activité et au pronostic. Les cas "actifs" avec expectoration constamment négative pour le B.K. se sont mieux comportés que les cas "actifs" avec expectoration positive pour le B.K.

Les malades dont l'hospitalisation était prolongée ont été plus améliorés que ceux ayant été traités pendant moins de six mois. Chez 485 sujets, il y a eu des rechutes pendant le service militaire et encore plus fréquemment après la libération. Parmi la totalité des rechûtes observées, approximativement 96% survinrent pendant les trois premières années après la fin du traitement de la phase active de la tuberculose durant la période militaire. Les rechutes comprenaient pratiquement toutes les formes de la tuberculose intra et extra-thoracique. En septembre 1949, à la fin de la période d'observation de 625 individus atteints de tuberculose à minima en activité au moment du premier examen effectué par cette Section de Recherche, 370 étaient bien portants, 228 avaient une tuberculose en activité, il y avait 16 décès imputables à la tuberculose et 11 à d'autres causes. Parmi 342 cas de tuberculose *a minima* stabilisée, 262 étaient bien portants, 72 avaient une tuberculose en activité, il y avait 4 décès imputables à la tuberculose et 4 à d'autres causes. Parmi les 342 cas classifiés "stabilisés" au premier examen, 75 sujets avaient eu des rechûtes et une tuberculose en activité, 23 d'entre eux pendant le service militaire et 52 après la libération. Parmi les 20 malades qui succombèrent à une tuberculose évolutive, une aggravation précoce s'était produite dans 18 cas et 19 malades ont présenté une dissémination extra-thoracique des lésions à un moment donné, habituellement dans les douze mois succédant

à l'apparition de l'affection initiale. Parmi les 15 dont le décès est imputable à d'autres causes que la tuberculose, 10 avaient également eu des rechutes de l'affection.

En bref, parmi les sujets pour lesquels le diagnostic de tuberculose en activité avait été porté au moment du premier examen clinique par la Section de Recherche de l'Armée, 59% étaient vivants et bien portants à la fin de la période d'observation, par comparaison aux 77% obtenus dans les cas où le diagnostic de tuberculose paraissant stabilisée avait été porté.

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THE DRUG TREATMENT OF NONHOSPITALIZED PATIENTS WITH TUBERCULOSIS [1,2]

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INTRODUCTION

Since July, 1953, antimicrobial drugs have been used regularly in the treatment of tuberculous patients attending the chest clinics of the New York City Department of Health. The results at the end of six months in the first 348 patients treated with oral medication have been reported previously (1). Roentgenographic improvement occurred in 39 per cent, and reversal of infectiousness (as determined by sputum culture) was demonstrated in 52 per cent of all patients who received isoniazid and PAS for at least four months. The number of cases was too few and the period of study was too short, however, to justify conclusions as to the long-range effectiveness of the program. It seemed advisable, therefore, to review the departmental experience with all patients with active pulmonary tuberculosis who received drug treatment during the first year and with the small group which has now been observed for at least two years. The findings are the subject of this paper.

RESULTS

Twelve-Month Follow-Up Observations

From July, 1953, to July, 1954, 1,646 patients with active pulmonary tuberculosis were started on antimicrobial therapy. This total includes the 494 patients referred to in the earlier report. Of this total, 483 had their treatment discontinued before they had received drugs for at least four months and were eliminated from the study for the reasons given in table 1.

The most common reason for elimination from the group was admission to a tuberculosis hospital. The 224 patients so eliminated reflect the steadily improving hospital-bed situation during this period which made it unnecessary to treat patients for any great length of time while awaiting admission to an institution. The next largest category, patients who failed to attend the clinic regularly, included 148 patients, or less than 9 per cent of the total of treated patients, and was substantially smaller than in earlier studies. Toxic reactions were given as the reason for complete discontinuance of drug therapy in only 18 patients. A change in drug therapy and the employment of a combination of isoniazid, streptomycin, and PAS which was more easily tolerated made it possible to complete the period of treatment in all other instances.

The remainder of this section of the paper deals with the findings at the end of twelve

[1] From the Bureaus of Tuberculosis and Laboratories, New York City Department of Health.

[2] Presented, in part, at the meeting of the New York State Academy of Preventive Medicine at the Hotel Statler, New York City, May 9, 1956.

TABLE 1

PATIENTS WITH ACTIVE PULMONARY TUBERCULOSIS STARTED ON DRUG THERAPY IN HEALTH DEPARTMENT CLINICS DURING TWELVE-MONTH PERIOD, JULY, 1953–JUNE, 1954
Number Removed and Number Remaining for Analysis After Twelve Months of Treatment

	Number	Per Cent		
Total started on treatment during twelve-month period				1,646
Discontinued before completion of four months of treatment			483	
Maximal benefit	5	1		
Toxicity	18	4		
Admitted to hospital	224	46		
Treatment elsewhere	22	5		
Died from tuberculosis	15	3		
Died from other causes	6	1		
Uncooperative	148	31		
Other reasons	45	9		
No report available at twelve months			17	
Total removed				500
Number remaining in study				1,146

months of observation in the 1,146 patients who received drug treatment for four months or more which was started between July, 1953, and July, 1954.

Three different drug regimens were employed: isoniazid-PAS, isoniazid-streptomycin, and isoniazid-streptomycin-PAS. Seventy-four per cent of the patients received isoniazid-PAS; 2 per cent, isoniazid-streptomycin; and 1 per cent, isoniazid-PAS-streptomycin exclusively. The remaining 23 per cent were given various combinations of the three regimens used. The daily dose of isoniazid was 5 mg. per kg. of body weight. Streptomycin, when used, was administered in 1 gm. doses twice weekly, and 12 gm. of PAS per day was prescribed. It was not feasible to supervise the taking of oral drugs, to check on the actual intake of PAS, or to make spot determinations of serum or urine isoniazid concentrations. The basic characteristics of this group are indicated in tables 2 and 3.

The age, sex, and color distribution of the group is shown in table 2. The majority of the patients were less than forty-five years of age as a result of the large number of young

TABLE 2

AGE, SEX, AND COLOR DISTRIBUTION OF 1,146 PATIENTS STARTED ON ANTIMICROBIAL THERAPY, JULY, 1953–JUNE, 1954

Age Group	Total			White			Non-White		
	Total	Male	Female	Total	Male	Female	Total	Male	Female
Less than 25*	96	41	55	55	28	27	41	13	28
25–34	255	116	139	116	57	59	139	59	80
35–44	258	154	104	128	76	52	130	78	52
45–54	229	185	44	140	118	22	89	67	22
55–64	187	164	23	139	123	16	48	41	7
65 and older	121	102	19	105	90	15	16	12	4
Total	1,146	762	384	683	492	191	463	270	193

* Less than 25 includes 2 aged 10, 3 aged 13, rest of group falls in 15–24 year bracket.

TABLE 3

CHARACTERISTICS OF 1,146 PATIENTS WITH ACTIVE PULMONARY TUBERCULOSIS AT START OF DRUG TREATMENT

	Number	Per Cent
Stage of disease		
Minimal	143	12
Moderately advanced	593	52
Far advanced	410	36
Duration of disease		
Less than one year	319	28
One to 5 years	506	44
More than 5 years	321	28
History of hospitalization		
None	232	20
More than 6 months previously	505	44
Less than 6 months previously	409	36

females who were included. This was true despite the fact that the males, who made up two-thirds of all cases, were older, and 76 per cent of the 207 white males were more than forty-five. One hundred and fifty-three persons born in Puerto Rico are represented in the white total of 683 patients.

In table 3 data are presented on several important factors in the history of these 1,146 patients prior to their inclusion in the program. Twenty-eight per cent were known to the Department of Health as having had tuberculosis for less than a year; 44, one to five years; and the other 28 per cent for more than five years, before their drug treatment in the clinic was started. Only 232, or 20 per cent of the patients, had never been hospitalized previously. The remainder gave a history of one or more periods of hospitalization. More than one-third of all of the patients with active pulmonary tuberculosis which had been treated had left an institution within six months of the time therapy was started.

All patients were advised to rest as much as was consistent with the routines they followed at home, carried out their usual domestic responsibilities, and made periodic visits to the clinic. Regulation or close supervision of their activities was not attempted. No patient received collapse or excisional therapy during the period of clinic observation.

Bacteriologic results: The changes in the sputum status of these 1,146 patients at the end of twelve months of observation are shown in table 4. Tubercle bacilli could no longer be recovered on culture in 56 per cent of the patients who were positive at the beginning of therapy. Only 3 per cent of the patients whose sputum was originally negative for tubercle bacilli had become positive at the end of this time. Reversal of infectiousness occurred in 76 per cent of the 277 patients with tubercle bacilli in the sputum who had never received drug treatment previously. The appearance of isoniazid-resistant strains of tubercle bacilli (defined as any growth following direct inoculation on media) was studied in 334 patients whose organisms were susceptible to isoniazid prior to antimicrobial therapy.

TABLE 4

SPUTUM STATUS AT TWELVE MONTHS OF 1,146 PATIENTS WITH ACTIVE PULMONARY TUBERCULOSIS AT START OF DRUG TREATMENT

Culture Report at Start	Total	Culture Report at Twelve Months			
		Positive	Negative	No Report	Per Cent Converted
Positive	720	282	366	72	56
Negative	207	5	160	42	3
No report	219	21	130	68	

TABLE 5

ROENTGENOGRAPHIC STATUS AT TWELVE MONTHS OF 1,146 PATIENTS WITH ACTIVE PULMONARY TUBERCULOSIS BY STAGE AT START OF DRUG TREATMENT

Stage at Start of Treatment	Total Number*	Roentgenographic Status at Twelve Months					
		Improvement		No Change		Deterioration	
		Number	Per Cent	Number	Per Cent	Number	Per Cent
Minimal	143	86	67	42	32	1	1
Moderately advanced	593	250	45	239	43	65	12
Far advanced	410	159	42	153	41	64	17
All patients	1,146	495	47	434	41	130	12

* Includes 87 patients with no roentgenogram available at twelve months.

Twelve months after the start of therapy tubercle bacilli were recovered from 110 patients and, in 50 (45.4 per cent), isoniazid-resistant strains were present. This represents the appearance of resistance to isoniazid in 15.0 per cent of the originally infectious and drug-susceptible cases at the end of one year.

Roentgenographic results: Roentgenographic improvement was noted in almost half of all cases. As might be anticipated, favorable changes occurred more frequently in the minimal lesions and in patients with more recent disease and no history of previous antimicrobial therapy. Roentgenographic deterioration occurred in 12 per cent of all patients under treatment (table 5).

TABLE 6

CLASSIFICATION OF DISEASE AT TWELVE MONTHS OF 1,146 PATIENTS WITH ACTIVE PULMONARY TUBERCULOSIS BY STAGE AT START OF DRUG TREATMENT

Stage at Start of Treatment	Total	Classification at Twelve Months					
		Number			Per Cent		
		Active	Arrested	Arrested with Cavity	Active	Arrested	Arrested with Cavity
Minimal	143	37	104	2	26	73	1
Moderately advanced	593	331	236	26	56	40	4
Far advanced	410	329	60	21	80	15	5
All patients	1,146	697	400	49	61	35	4

Change in over-all status after one year of chemotherapy: The results of the program in terms of changes in clinical classification are given in table 6. In 400 of the 1,146 patients (35 per cent), the tuberculosis met the National Tuberculosis Association criteria for arrest. In an additional 4 per cent stable roentgenographic findings and sputum cultures persistently negative for tubercle bacilli were present in association with localized rarefaction on the chest film. Patients with less advanced or more recent disease and no history of previous treatment did substantially better than those with more extensive lesions who had failed to respond to drug therapy in the past.

Twenty-Four-Month Follow-Up Observations

As part of the evaluation of the long-range results of antimicrobial therapy, two-year follow-up data have been collected on the 677 patients with active pulmonary tuberculosis started on treatment between July and October, 1953. Three hundred and fifty-one of these patients completed a minimum of eighteen months of chemotherapy or reached the stage of maximal benefit before that time had elapsed. Therapy was discontinued in the remainder for a variety of reasons including admission to a hospital or failure to keep clinic appointments. Most of these discontinuances occurred shortly after treatment was started; 60 per cent occurred in the first six months.

The composition of this group of 351 patients was less favorable than that of the 1,146 patients treated during the entire first year of the program, as shown in table 7. More than half of the former were more than forty-five years of age, and in only 8 per cent was the disease in the minimal stage at the start of drug therapy. Only 19 per cent had been discovered to have tuberculosis for the first time less than a year previously, and one of 4 had never been hospitalized for tuberculosis. These differences are due to the inclusion of a larger proportion of long-term clinic patients in the group treated during the first three months of the program than subsequently.

Bacteriologic and roentgenographic results: In 256 of the total of 351 patients

TABLE 7

CHARACTERISTICS OF 351 PATIENTS WITH ACTIVE PULMONARY TUBERCULOSIS AT START OF DRUG TREATMENT FOR WHOM A REPORT WAS AVAILABLE AT TWENTY-FOUR MONTHS

	Number	Per Cent
Stage of disease		
Minimal	29	8
Moderately advanced	171	49
Far advanced	151	43
Duration of disease		
Less than one year	66	19
One to 5 years	147	42
More than 5 years	138	39
History of hospitalization		
None	86	25
More than 6 months previously	191	54
Less than 6 months previously	74	21

sputum cultures were positive for tubercle bacilli at the time treatment was begun, and in 140 (58 per cent) the cultures were negative twenty-four months later. In no instance was a positive culture obtained at the end of the twenty-four months from a patient whose initial sputum examination was negative for tubercle bacilli. Reversal of infectiousness occurred in a higher percentage of the patients who had not received antimicrobial therapy previously and of those with less advanced disease.

Similar findings were noted in regard to roentgenographic changes, with improvement occurring in 47 per cent and deterioration in 12 per cent of the patients treated for the two-year period. In general, the minimal cases and the patients previously untreated fared better, in agreement with the results at the one-year observation point (table 8).

Change in over-all status after two years of chemotherapy: The clinical classification at the end of twenty-four months of all patients with active pulmonary tuberculosis at the time treatment was started is presented in table 9. In 48 per cent of the patients, the tuberculosis had become arrested by the 1950 National Tuberculosis Association standards, and in an additional 12 per cent the disease could be placed in the category of "arrested with residual cavitation" described earlier in this report. Only 40 per cent of the patients had lesions which were still considered to be active. After two years of treatment, the disease could be classified as arrested in 83 per cent of the minimal cases and in 42 per cent of

TABLE 8

SPUTUM STATUS AT TWENTY-FOUR MONTHS OF 351 PATIENTS WITH ACTIVE PULMONARY TUBERCULOSIS AT START OF DRUG TREATMENT

Culture Report at Start	Total	Culture Report at Twenty-Four Months			
		Positive	Negative	No Report	Per Cent Converted
Positive	256	100	140	16	58
Negative	53	—	47	6	—
No report	42	5	34	3	

TABLE 9

CLASSIFICATION OF DISEASE AT TWENTY-FOUR MONTHS OF 351 PATIENTS WITH ACTIVE PULMONARY TUBERCULOSIS BY STAGE AT START OF DRUG TREATMENT

Stage at Start of Treatment	Total	Classification at Twenty-Four Months					
		Number			Per Cent		
		Active	Arrested	Arrested with Cavity	Active	Arrested	Arrested with Cavity
Minimal	29	5	24	—	17	83	—
Moderately advanced	171	50	103	18	29	60	11
Far advanced	151	87	40	24	58	26	16
All patients	351	142	167	42	40	48	12

TABLE 10

SPUTUM STATUS AT TWELVE AND TWENTY-FOUR MONTHS OF 256 PATIENTS WITH INFECTIOUS SPUTUM AT START OF TREATMENT

		Status at Twenty-Four Months with Respect to *M. Tuberculosis*		
		Positive	Negative	No Report
Total	256	100	140	16
Positive	100	82	12	6
Negative	150	15	126	9
No report	6	3	2	1

the patients with far advanced disease. Patients who had not received drug therapy previously showed greater improvement than those who had been treated in the past. A comparison of the bacteriologic status at the end of twelve and twenty-four months of observation was possible in the 256 patients with tubercle bacilli in the sputum at the time treatment was begun. At the end of one year reversal of infectiousness had occurred in 150 of the patients whose sputum originally had contained tubercle bacilli. Twelve months later 126 were still noninfectious, and no report was available in 9, indicating a persistence of the noninfectious state in 89 per cent of the cases. In addition, tubercle bacilli could no longer be recovered at the end of two years from the sputum of 100 patients who were still discharging bacilli after one year of chemotherapy, a "late conversion rate" of almost 13 per cent (table 10).

DISCUSSION

An analysis of the results obtained in a large group of unhospitalized patients who received tuberculosis chemotherapy for periods up to two years makes possible a realistic appraisal of the accomplishments of the program from both the clinical and public health points of view. Clinical improvement occurred in a large number of patients whose earlier treatment was unsuccessful. This was demonstrated by roentgenographic clearing and reversal of infectiousness; in a substantial proportion of the group the disease could be classified as arrested. Patients with less extensive and more recent disease who had received no previous chemotherapy did better than patients with advanced tuberculosis of long standing which had failed to respond to drugs in the past. These favorable effects, which were most marked during the first six months of therapy, occurred in an increasing number of cases during the remainder of the two-year period. Continued observation would indicate that deterioration has been checked in patients awaiting admission to a hospital and the need for institutional treatment obviated in the 60 per cent whose disease stability has been demonstrated at the end of two years.

The public health results have been equally impressive. Improvement has been shown by disappearance of tubercle bacilli in the sputum of 58 per cent of the 256 originally infectious patients. In addition, the entire behavior of these patients has become more cooperative. During the first twelve months, treatment was

discontinued in only 8 per cent and in twenty-four months in only 15 per cent of patients because of their irregularity in clinic attendance. The doctors and nurses caring for these patients in the clinic and at home report a higher level of cooperation and observance of medical advice. This they attribute to the patients' conviction that something tangible is being done to help them.

The most valid measure of the success of such a program from the public health point of view is the proportion of the entire group of unhospitalized patients whose disease can be considered stable and noninfectious two years after the start of treatment. In arriving at such a figure, one must include in the total all patients who lapsed from clinic attendance while their disease was still active and those hospitalized for tuberculosis after an adequate period of antimicrobial therapy in the clinic. On this basis 36.7 per cent of the patients given drugs for at least four months could be classified as having attained arrest of their disease at the end of two years of observation. By applying similar criteria, 49.0 per cent could be considered to have converted their sputum to a noninfectious state during this period.

Certain unfavorable features have also been noted in connection with the drug treatment of nonhospitalized patients with tuberculosis. Progression of the disease occurred in 12 per cent of all patients. Moreover, tubercle bacilli were still present in the sputum of 100 of the 256 originally infectious patients after twenty-four months of antimicrobial therapy. Sixteen patients died of tuberculosis during the period of observation. In addition to these objective clinical findings, several theoretic criticisms of the program have been advanced. One is that the administration of drugs to nonhospitalized patients encourages them to remain out of tuberculosis hospitals. A study of the writers' material indicates that this criticism does not apply to the first admission of a tuberculosis patient. Only 12 per cent of the new cases diagnosed in the clinics of the Department of Health in a one-year period were started on chemotherapy in the clinic, and many of these were awaiting hospital admission at the time. The remaining 88 per cent were referred for treatment to hospitals or private practitioners.

Another objection raised is that the availability of drug therapy in clinics induces patients to leave institutions prematurely. This is not borne out by the records. Twenty-six per cent of all discharges were against medical advice in New York City hospitals in 1954 when the Health Department program was in full operation, compared with 30 per cent in 1952, the year before it was started (2). There is also no evidence that the frequency with which the patient returned to the hospital for further care has been materially affected. More than half of the patients with active tuberculosis in the city were segregated in institutions at the end of 1954, not too different a figure from earlier years or from other areas where such treatment was not available to nonhospitalized patients (3).

Unsanctioned discharge from tuberculosis institutions and the reasons for it have been the subject of several recent papers (4,5). These studies have shown that the basic factors responsible for the patient's leaving have been dissatisfaction with institutional life, and concern over family and social problems outside the hospital. The writers' experiences confirm this and indicate that these

same influences, rather than the opportunity of obtaining drugs in the clinic, are the primary cause of difficulty in persuading patients to re-enter institutions for further treatment. That is one of the reasons why the medical social worker and vocational rehabilitation counselor have been made members of the clinic staff. It is hoped that by combining the resources of physician, public health nurse, medical social worker, and rehabilitation counselor as a team, many of the social and economic problems of patient and family can be minimized, if not eliminated.

One other possible result of this type of program has been a matter of considerable concern: the spread of strains of tubercle bacilli resistant to the common antimicrobial drugs. This theoretically could happen if a substantial number of unhospitalized patients had such organisms in their sputum, whether their treatment was originally given in the hospital or in the clinic. Resistance to isoniazid of tubercle bacilli in the sputum of patients under clinic care has been repeatedly determined and found to have developed at the end of one year in 6 per cent of the previously untreated and 22 per cent of the previously treated initially drug-susceptible cases. Another approach has been by analysis of the susceptibility to streptomycin and to isoniazid of organisms recovered from the sputum of patients with tuberculosis newly reported by clinics and hospitals in New York City. Such a study, recently reported by Chaves and associates (6), has shown significant primary resistance was present in approximately 2 per cent during the year 1955.

Summary

The results of antimicrobial therapy at the end of one year have been analyzed in the 1,646 unhospitalized patients with active pulmonary tuberculosis who were started on treatment in the chest clinics of the New York City Department of Health between July 1, 1953, and June 30, 1954.

One thousand one hundred and forty-six (70 per cent) of the entire group received therapy for at least four months. Most of those who did not complete the four-month period were admitted to a hospital or discontinued regular clinic attendance.

Reversal of infectiousness as determined by sputum culture occurred at the end of twelve months in 56 per cent of the patients with sputum initially positive for *M. tuberculosis*. Only 3 per cent of the patients with sputum initially negative for *M. tuberculosis* were "positive" at this time. Reversal of infectiousness occurred in 44 per cent of the patients who had received, and 76 per cent of the patients who had not received, antimicrobial drugs prior to inclusion in the program.

Roentgenographic improvement was observed in 47 per cent of all cases, and deterioration in 12 per cent, after one year of treatment. Thirty-five per cent of the group met more rigid criteria for arrest than those of the 1950 *Diagnostic Standards* of the National Tuberculosis Association. Four per cent with consistently negative bacteriologic findings and stable roentgenograms showed localized areas of rarefaction on the films and were classified as "arrested with cavitation."

The stages of arrest and arrest with cavitation were reached in 43 and 3 per cent, respectively, of the patients who had not received drug treatment previously.

Clinic treatment was most successful in patients with minimal disease of recent origin which had not previously been treated with antimicrobial drugs.

Study of the findings in 351 patients with active pulmonary tuberculosis who were started on drug therapy between July and October, 1953, indicates that the improvement noted in the first year of treatment was persistent and was present in an even greater proportion of cases at the end of twenty-four months.

Tubercle bacilli could no longer be recovered on culture in 58 per cent of the patients in whose sputum they were demonstrated at the beginning of treatment.

The proportion of cases classified as arrested according to the criteria of the National Tuberculosis Association rose to 48 per cent; and of those arrested with cavitation, to 12 per cent after an additional year of observation. More favorable responses were obtained in the less advanced, more recent, previously untreated cases, as noted earlier.

An over-all appraisal of the results of the program indicates that its accomplishments have been substantial from both clinical and public health points of view. Two years after the start of treatment, only 32 per cent of the 351 patients had tubercle bacilli in the sputum, and in no less than 37 per cent the disease could be classified as "arrested." These results obtained even when patients admitted to a hospital or failing to attend clinic regularly were included in the total.

The writers' experience provides no evidence that the provision of drugs to unhospitalized patients through clinic services has materially influenced acceptance of hospitalization or contributed to the spread of tubercle bacilli resistant to antimicrobials in the community.

Sumario

La Farmacoterapia de los Tuberculosos No Hospitalizados

Analízanse aquí los resultados de la terapéutica antimicrobiana al cabo de un año en los 1,646 enfermos de tuberculosis pulmonar activa, no hospitalizados, en quienes iniciaron tratamiento las clínicas torácicas del Departamento de Sanidad de la Ciudad de Nueva York entre el 1° de julio de 1953 y el 30 de junio de 1954.

Mil ciento cuarenta y seis (70 por ciento) de todo el grupo recibieron terapéutica a lo menos cuatro meses. La mayor parte de los que terminaron el período de cuatro meses ingresó en un hospital o abandonó la asistencia fija a las clínicas.

En 56 por ciento de los enfermos cuyo esputo era inicialmente positivo para el *M. tuberculosis* hubo viraje del esputo en los cultivos al cabo de doce meses. Sólo 3 por ciento de aquéllos cuyo esputo era inicialmente negativo para el *M. tuberculosis* era positivo para dicha fecha. Ocurrió viraje de la infecciosidad en 44 por ciento de los enfermos que habían recibido y 76 por ciento de los que no habían recibido drogas antimicrobianas antes de formar parte del estudio.

Observóse mejoría radiográfica en 47 por ciento de todos los casos, y deterioro en 12 por ciento, al cabo de un año de tratamiento. Un 35 por ciento del grupo cumplió pautas más rígidas de estacionamiento que las de las *Patrones de Diagnóstico* de 1950 de la Asociación Nacional contra la Tuberculosis de los E. U. A. Un 4 por ciento que mostró hallazgos bacteriológicos constantemente negativos y radiografías estables reveló zonas localizadas de

enrarecimiento en las radiografías y fué clasificado como "estacionado con cavernas." Alcanzaron los períodos de estacionamiento y estacionamiento con cavernas 43 y 3 por ciento, respectivamente, de los enfermos que no habían recibido farmacoterapia anteriormente.

El tratamiento en la clínica logró su mayor éxito en los sujetos que tenían enfermedad mínima de iniciación reciente y no habían sido tratados antes con drogas antimicrobianas.

El estudio de los hallazgos en 361 enfermos de tuberculosis pulmonar activa en quienes se inició la farmacoterapia entre julio y octubre de 1953 indica que la mejoría notada en el primer año de tratamiento persistía y abarcaba una proporción aun mayor de casos al cabo de veinticuatro meses.

Al cabo del período, ya no podían obtenerse bacilos tuberculosos en los cultivos in 58 por ciento de los enfermos en quienes se habían encontrado al comenzar el tratamiento.

La proporción de casos clasificados como estacionados conforme a las pautas de la Asociación Nacional contra la Tuberculosis se elevó a 48 por ciento, y la de los estacionados con cavernas, a 12 por ciento al cabo de un año más de observación. Según se señaló antes, las respuestas más favorables fueron en los casos menos avanzados, más recientes y no tratados previamente.

Una justipreciación global de los resultados de la obra indica que sus realizaciones han sido considerables desde los puntos de vista tanto clínico como sanitario. A los dos años de la iniciación del tratamiento, sólo 32 por ciento de los 351 enfermos tenían esputo positivo para *M. tuberculosis* y no menos de 37 por ciento estaban clasificados como "estacionados," aun cuando el total comprendía a los enfermos recibidos en hospitales o que no asistían con regularidad a las clínicas.

Las observaciones de los AA. no aportan datos de que el suministro de drogas a los enfermos no hospitalizados haya afectado mayor cosa la aceptación de la hospitalización o contribuído a la difusión en la colectividad de bacilos tuberculosos resistentes a las drogas antimicrobianas.

Resume

Traitement antibactérien du tuberculeux non hospitalisé

Les résultats du traitement antibactérien au bout d'une année ont été analysés chez les 1.646 malades atteints de tuberculose pulmonaire active, traités sans hospitalisation dans les cliniques des Maladies thoraciques du Département d'Hygiène publique de New York City entre le ler juillet 1953 et le 30 juin 1954.

Parmi le groupe tout entier, 1.146 malades (70%) ont été soumis à cette therapeutique pendant au moins quatre mois. La plupart de ceux n'ayant pas effectué la cure complète de quatre mois entrèrent à l'hôpital ou cessèrent les visites régulières a la clinique.

La négativation des cultures de crachats est manifestée dans 56% des cas au bout des douze mois chez les malades dont l'expectoration était initialement positive pour *le M. tuberculosis*. Il n'y eut que 3% des malades dont les crachats étaient initialement négatifs qui présentèrent des crachats positifs au bout de cette période. La disparition de la contagiosité apparaissait chez 44% des malades soumis au traitement par les agents antibactériens et 76% de ceux qui n'avaient pas été traités par ces agents avant leur participation au programme.

L'amélioration des aspects radiologiques fut observée dans 47% de la totalité des cas et l'aggravation dans 12% des cas au bout d'un an de traitement. Dans ce groupe 35% des cas ont satisfait aux critères de stabilisation qui étaient plus rigides que ceux des *Standards diagnostiques* de 1950 de la *National Tuberculosis Association*. Des examens bactériologiques constamment négatifs et des radiogrammes non modifiés montrant des zones localisées de raréfaction du parenchyme furent observés dans 4% des cas qui sont classifiés "stabilisation avec excavation." Le stade de stabilisation et de stabilisation avec excavation a été atteint par 43% et par 3%, respectivement, des malades n'ayant pas été soumis antérieurement à cette thérapeutique.

C'est chez les malades atteints de tuberculose a minima d'origine récente, n'ayant pas été antérieurement traités par les antibiotiques que le traitement a été le plus satisfaisant.

L'analyse des résultats chez 351 malades atteints de tuberculose pulmonaire en activité, qui furent pour la première fois soumis au traitement antibactérien entre juillet et octobre 1953, indique que l'amélioration observée dans la première année du traitement persistait et se maintenait dans une proportion des cas encore plus grande au bout de vingt-quatre mois.

Les bacilles tuberculeux n'étaient plus recouvrés dans les cultures chez 58% des malades dont l'expectoration était positive au début du traitement.

La proportion des cas classifiés "stabilisés" selon les normes de "National Tuberculosis Association" s'est élevée à 48%, et les cas "stabilisés avec excavation" à 12% après encore une autre année d'observation. Comme il a déjà été mentionné, les résultats les meilleurs s'obtinrent dans les cas les moins avancés, les plus récents et non traités antérieurement.

Une évaluation d'ensemble des résultats du programme indique que ses réalisations furent importantes tant cliniquement que du point de vue de l'hygiène publique. Deux ans après la mise en oeuvre du traitement, il n'y avait, sur un nombre de 351 malades, que 32% des cas où le sputum était positif pour le bacille tuberculeux, et les cas classifiés "stabilisés" atteignaient au moins 37%, même en comprenant dans le total les sujets admis à l'hôpital ou qui ne se présentaient pas régulièrement à la clinique.

Selon l'expérience des auteurs, il n'est pas démontré que l'allocation des antibiotiques aux malades non hospitalisés, par l'intermédiaire de la clinique, ait eu une influence sur l'acceptation de l'hospitalisation ou qu'elle ait contribué à accroître la résistance des bacilles dans la région.

Acknowledgment

This study would have been impossible without the untiring efforts of all of the medical and nursing staff of the Bureaus of Tuberculosis and Nursing and of many members of the Bureaus of Laboratories and Records and Statistics of the Department of Health.

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PULMONARY CYSTS[1]

Five Cases with Preoperative and Postoperative Pulmonary Function Studies

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INTRODUCTION

It is often difficult to determine the proper management of the symptomatic patient with a pulmonary cyst. The problem is easier when a giant bleb or bulla exists. Cournand and others have elucidated the various pathophysiologic respiratory patterns caused by these enormous cysts (1), and numerous articles attest to the improvement that may be expected in many of the patients following surgical therapy (2–5). There remains, however, a group of patients who present themselves because of symptoms, usually shortness of breath, with a lung cyst of moderate size. The literature contains several reports on excisional surgery of such cysts, but none contains preoperative and postoperative pulmonary function studies. This present report consists of a review of 5 patients with pulmonary cysts who were evaluated with ventilatory studies preoperatively and postoperatively.

OBSERVATIONS

Case 1. A 37-year-old man had left-sided pleurisy ten and eight years before admission. Three years previously he had a spontaneous hemothorax on the left, and hemoptysis in varying amounts had been intermittent since. Mild cough, scant mucoid sputum, and exertional dyspnea had been prominent symptoms for several years. The expiratory phase of respiration was prolonged. The chest films are shown in figures 1*A* and *B*. Fluoroscopic examination was normal except for some displacement of the mediastinum by the cyst. Table 1 contains the preoperative pulmonary function data. A multiloculated emphysematous bleb in the left upper lobe was resected; no other blebs were noted. Eleven months postoperatively the function studies were repeated (table 1). There had been only minimal improvement in the shortness of breath.

Comment: The low diaphragms and increased radiolucency in the roentgenogram indicated generalized bronchopulmonary disease. The lack of significant clinical improvement following removal of the cyst indicated that the cyst was not contributing very much to the patient's disability. The mild reduction in total vital capacity before surgery was unchanged postoperatively; there was less obstructive defect, however, as shown by the increase in vital capacity (3 seconds)/vital capacity (total) from 70 to 87 per cent. This cannot necessarily be ascribed to the surgery since wide variations in timed vital capacity measurements occur from day to day in these patients. The oxygen consumption difference between the two lungs suggests the possibility of an abnormal vascular supply to the right lung. Operation was followed by pleural reaction (figure 1*B*). One wonders how much difference there was between the function gained by

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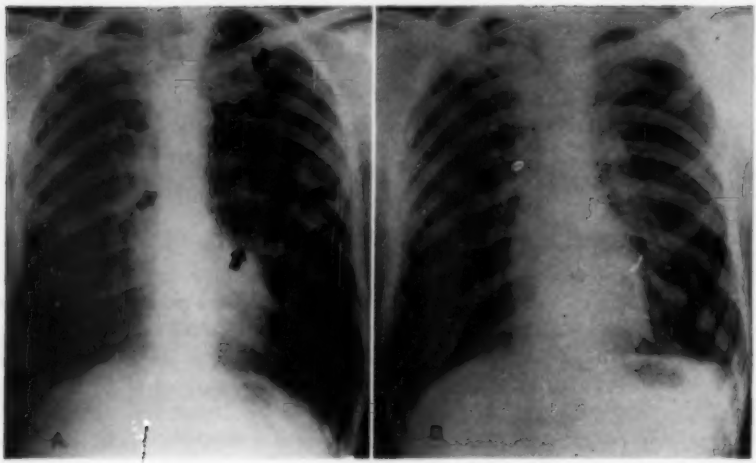


Case 1

FIG. 1*A*. A large cyst (arrows) is present in the left upper lobe in this preoperative film. It extends into the mediastinum, displacing it slightly. There is scattered fibrosis. *B*. Film obtained twenty months postoperatively.

removal of the cyst and the function lost because of the pleural reaction. There would be considerable reluctance about operation if this case were being considered now instead of three years ago.

Case 2. A 42-year-old man sought hospital admission because his private physician had advised him to have surgical removal of a "cyst in his lung." He had had chronic cough with mucoid sputum for many years, and shortness of breath had been present for nine months. No weight loss, wheezing, chest pain, or hemoptysis were observed. Breath sounds were diminished over the left upper lung and sonorous rales were heard throughout the right chest. The chest film is shown in figure 2*A*. Chronic bronchitis only was seen at bronchoscopy; cell block of the washings was negative for tumor. Pulmonary function data are recorded in table 1.

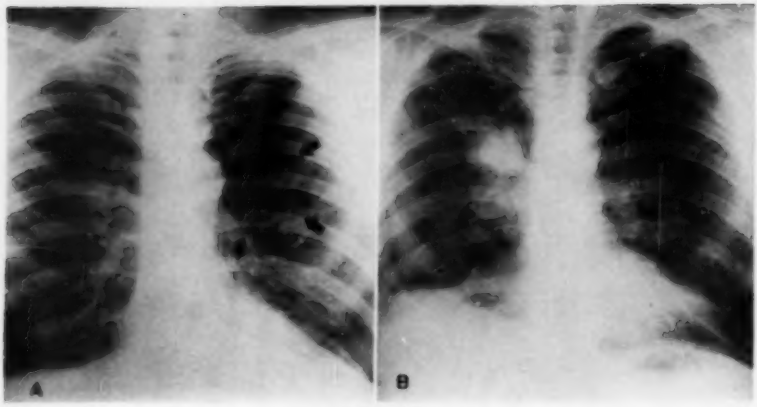
Resection of the bulla on the left was performed. Large blebs over the lingular, apical, and posterior portions of the left upper lobe were resected. The remaining lung was emphysematous. The patient's symptoms were improved. Repeat pulmonary function testing was done ten weeks after surgery (table 1). Shortly thereafter the right hemithorax was explored, a squamous cell carcinoma was found, and right upper lobectomy was performed. The patient returned to work three weeks later and at follow-up four months later was asymptomatic except for mild shortness of breath on exertion. The tumor recurred after lobectomy (figure 2*B*).

Comment: Total vital capacity, 3-second vital capacity, and maximal breathing capacity were all reduced, indicating more pulmonary disease than could be explained by the bulla alone. In accord with this were the rales which were heard on the right side. Exploration on the right was postponed in the hope of improving the function on the left, but the data presented in table 1 show that this was not accomplished. Symptoms were thought to have been improved by the

TABLE 1

PULMONARY FUNCTION DATA OF 5 PATIENTS WITH PULMONARY CYSTS AT PREOPERATIVE AND POSTOPERATIVE STAGES

Pulmonary Function	Case 1		Case 2		Case 3			Case 4			Case 5		
	Preoperative (4/1952)	Postoperative (3/1953)	Preoperative (2/1953)	Postoperative (5/1953)	Preoperative (8/1953)	Postoperative (9/1953)	Postoperative (1/1954)	Preoperative (1/1953)	Postoperative (7/1953)	Postoperative (3/1954)	Preoperative (1/1953)	Postoperative (8/1953)	Postoperative (3/1955)
Minute volume (L)	6.83	8.26	7.98	12.9	11.3	8.36		8.51	8.52	9.80	7.76	7.46	8.28
Respiratory rate	12	12	13	12	22	23		17	12	11	11	11	11
Tidal volume (cc.)	570	683	633	1.040	504	360		506	710	891	706	692	781
Ventilation (L/Min./M2)													
Rest	3.9	4.9	3.6	6.1	6.6	4.9		4.3	4.3	5.0	3.8	3.9	4.1
Exercise	7.0	9.8	10.5	10.6	13.5	13.3		9.3	10.7	10.3	14.8	13.5	16.4
Oxygen consumption (cc./Min./M2)													
Rest	130	113	140	148	139	122		158	157	173	178	134	135
Exercise	465	490	597	575	698	560		882	879	610	792	812	412
Oxygen removal rate (cc./Min./L vent)													
Rest	32.5	22.8	38.3	24.2	20.5	25.1		36.6	36.2	34.8	46.4	35.6	32.7
Exercise	65.0	49.7	56.8	54.3	51.7	42.1		95.5	82.1	59.2	53.6	60.1	25.1
Inspiratory capacity (L)	2.54	2.32	2.59	1.98	1.72	1.92	1.87	3.75	4.01	3.84	2.83	2.63	2.78
Total vital capacity (L)	3.58	3.31	3.63	2.33	2.03	2.32	2.86	4.75	4.63	5.10	3.50	3.64	4.00
Three-second vital capacity (L)	2.54	2.88	2.50	2.18	1.25	1.65	1.82	3.82	3.87	4.79	2.61	2.89	2.84
Expiratory-respiratory volume (cc.)	1,025	990	1,180	1,410	812	735	1,170	1,360	1,300	1,410	846	1,200	1,090
Maximal expiratory pressure (mm./Hg.)	120	80	140	120	90	80		100	110		140	120	
Maximal breathing capacity (L/Min.)	150	154	111	110	50.8	55.7	48	148	150	148	96.4	82.3	114
Breathing reserve (per cent)	96	95	93	88	78	85		93	95		92	91	
Duration dyspnea (secs.)		55	60	80	75	50		65	75	70	65	55	65
Oxygen consumption (cc.)													
Right lung	54				225			156			114		
Left lung	102				124			187			256		



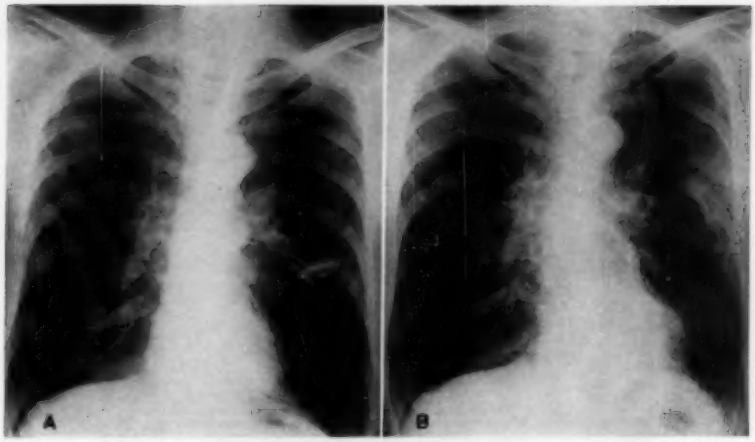
Case 2

FIG. 2A. Large cysts (arrows) occupy most of the left upper lung area in this preoperative film. A 3 cm. mass lies at the anterior end of the right third rib. *B*. Film obtained nineteen months following surgery. Recurrence of the tumor took place about the right hilum.

initial operation, but the psychologic response to surgery may be concerned here. It is believed that this case exemplifies poor judgment predicated on the assumption that the cyst was compromising the function of the left lung. There resulted a needless delay of three months in the treatment of cancer.

Case 3. A 56-year-old man had a long history of cough, purulent sputum, shortness of breath, and wheezing. Four years previously he was told he had a cyst in his left lung. The physical findings were those of bilateral emphysema and bronchospasm. Chest films are shown in figures 3*A* and *B*, and results of pulmonary function tests are shown in table 1. Surgical therapy was refused. Four months later the patient was hospitalized at another institution because of pneumonitis. His shortness of breath had increased in the four-month period and he requested transfer to the McKinney Veterans Administration Hospital when he had recovered from the pneumonia. At the time of his arrival there the roentgenographic findings were unchanged. Data on the status of his pulmonary function were compiled two weeks after the resection of a cyst in the left lower lobe. The patient was symptomatically improved after surgery, but this improvement was not accompanied by improvement in pulmonary function (*see* table 1). When last seen (fifteen months postoperatively) he had intermittent wheezing and shortness of breath, but thought that he had been benefited by surgery.

Comment: The cyst in the left lower lobe probably contributed to the symptoms of this patient. Pulmonary ventilatory values did not change following surgery except for the total vital capacity. The persistent reduction of the 3-second vital capacity was most certainly due to the generalized bronchitis and emphysema. Improvement had continued to occur as long as five months following operation; this suggests that final evaluation of function subsequent to any type of surgical procedure should be delayed at least five months after operation. Neither clinical nor physiologic improvement was very great in this man.



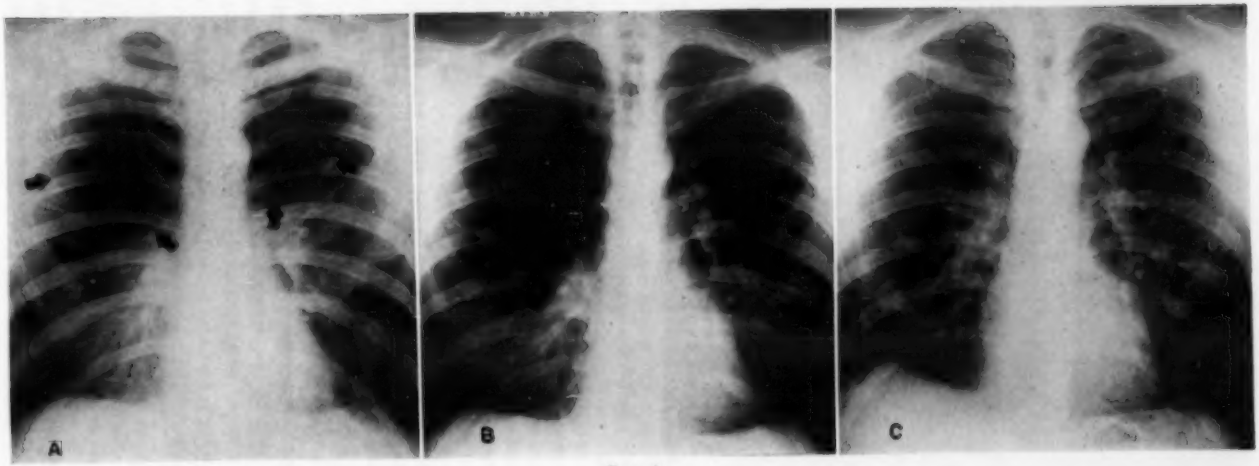
Case 3

FIG. 3*A*. A large cyst is seen in the left lower lobe of this preoperative film. *B*. Film obtained four months following surgery.

Case 4. The patient was a 35-year-old man with a six-month history of exertional dyspnea. The breath sounds were diminished over the upper lobes. His chest film is shown in figure 4*A*. Bronchoscopic examination was normal except for distortion of upper lobe bronchi by the bilateral blebs. Table 1 contains the pulmonary function data. Numerous emphysematous blebs were resected from the apical posterior portion of the left upper lobe and from the dorsal segment of the left lower lobe. The patient was much improved by surgery. Six months later pulmonary function was essentially unchanged from the preoperative values (table 1). The cysts on the right were the same size (figure 4*B*). Excision of the cysts on the right was done because of the persistence of some shortness of breath on exertion. Nine months later the patient was re-examined (table 1). He had no respiratory complaints when last seen eighteen months following operation (figure 4*C*).

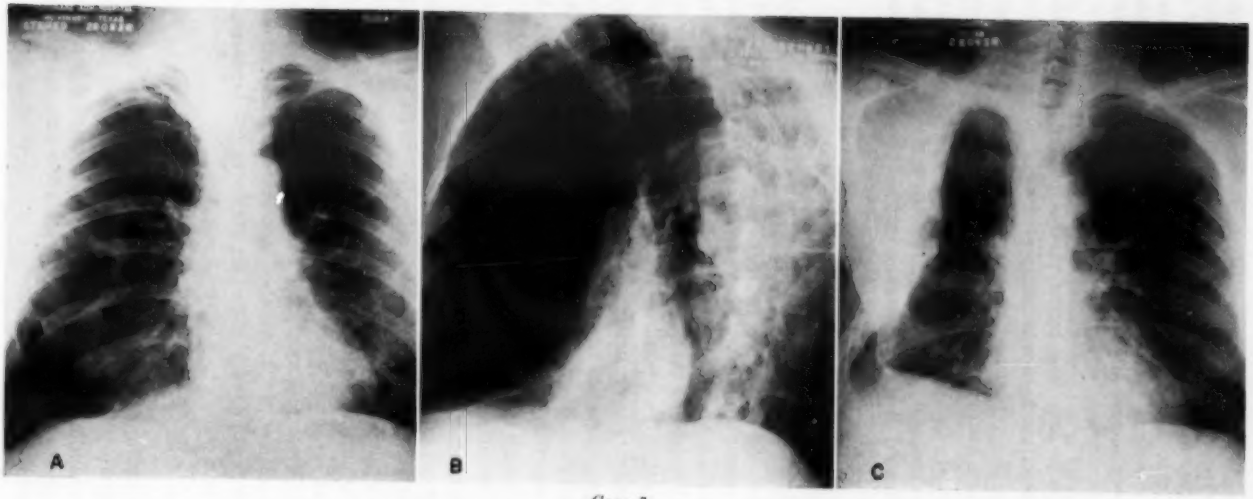
Comment: Cough and sputum were absent in this man, and the only symptom, exertional dyspnea, was of short duration. This is distinctly different from the 3 previous instances. The lack of any indication of generalized disease is of the utmost importance when a decision regarding surgical therapy is being made. The 3-second vital capacity/total vital capacity ratio was somewhat reduced (80 per cent), but this could be adequately explained by the compression of the lower lobes (figure 4*A*) and by the distortion of bronchi seen endoscopically. A slight delay in emptying would be anticipated in such a situation. Measurements made nine months following the second procedure revealed this ratio to be normal (94 per cent). This patient is an unequivocal therapeutic success.

Case 5. A 44-year-old engineer had been told six months before admission that he had bullous emphysema. Mild cough, scant sputum, and exertional dyspnea had been present for ten years. Tactile fremitus and breath sounds were diminished over the right chest. His chest films are shown in figures 5*A*, *B*, and *C*, and data on his pulmonary function are shown in table 1. Bronchograms revealed extrinsic pressure on the right upper lobe bronchi. At the time of surgery he was found to have several large cysts that occupied



Case 4

FIG. 4A. Preoperative film in which cysts are present in both upper lobes (arrows). The lungs below the cysts appear compressed. *B*. Film obtained six months following resection of the cysts on the left. *C*. Film obtained six months following resection of the cysts in the right upper lobe.



Case 5

FIG. 5.1. Several large cysts occupy the lateral part of the right hemithorax (arrows) in this preoperative film. *B*. Preoperative film of left anterior oblique view showing the area of the cysts. *C*. Film obtained six months following surgery.

most of the right upper lobe; this lobe with its cysts was resected. The surgeon chose to do a partial thoracoplasty in the hope of preventing overdistention of the remaining lobes. Six months later the patient was re-evaluated (table 1). He still had some shortness of breath, but thought that he was definitely better than before the surgery. Eighteen months later his symptoms were unchanged, but he was able to walk several miles daily, as well as climb numerous large oil tanks each day. The ventilatory studies are also shown in table 1.

Comment: The cystic area was larger in this patient than in the previous instances, and the situation here was interpreted as being intermediate between the other patients in this report and those studied by Cournand and associates (1). Again, there was a history of cough, sputum, and dyspnea for several years. Improvement followed operation, however, and would no doubt have been greater had not thoracoplasty been done. Resection of the cysts and the right upper lobe was indicated in spite of known chronic bronchitis and emphysema only because of the large size of the bullous area.

Discussion

The study of 5 patients is obviously inadequate to support any comments that would be definitive in the therapy of all patients with pulmonary air-containing cysts. The present report is unusual, however, in that it includes more complete ventilatory studies than have been published. Bronchodilators, antimicrobials, wetting agents, and breathing exercises were used vigorously prior to the measurement of lung function. Talc poudrage was performed in all patients: this may have affected postoperative ventilatory ability but was probably unimportant. Postoperative complications did not occur.

Only 2 of these patients (*Cases 4* and *5*) obtained any pronounced benefit from surgery. In *Case 4* this was due to the absence of chronic bronchitis and emphysema. In *Case 5*, although the patient had generalized bronchopulmonary disease, he also had a large cystic area. Patients with either of these situations can probably expect less dyspnea following removal of the blebs or bullae.

An entirely different condition existed in the first 3 patients. No more than slight improvement, clinically or physiologically, could be shown following surgery. They all had evidence of generalized bronchopulmonary disease by history or by physical or laboratory examination. In the presence of such disease there is no way to evaluate accurately just how much functional disturbance is due to the cyst. This inability is apparently the basis for the disappointing surgical results also noted by others (6, 7). Another important consideration is the size of the cyst. Only if the cyst is very large can improvement be expected routinely following surgical removal.

Summary

Five patients with lung cysts of moderate size were submitted to surgical therapy. The ventilatory function of the lung was measured preoperatively and for a suitable period following operation. The condition of 2 patients was considerably improved, whereas in the 3 other patients the surgical procedure was not followed by improvement.

Sumario

Cinco Casos de Quistes con Estudios Preoperatorios y Postoperatorios de la Función Pulmonar

Cinco enfermos con quistes pulmonares de tamaño moderado fueron sometidos a la terapéutica quirúrgica. La función ventiladora del pulmón fué medida preoperatoriamente y durante un período adecuado después de la intervención. El estado de 2 enfermos mejoró, sin que sucediera esto en los otros 3. Discútense las razones del fracaso.

Resume

Etude pré et post-opératoire de la fonction pulmonaire dans cinq cas

Cinq malades présentant des kystes pulmonaires de dimensions modérées furent soumis au traitement chirurgical. La fonction de ventilation pulmonaire fut déterminée avant l'intervention et pendant une période appropriée à la suite de l'opération. L'état de 2 malades était amélioré; celui de 3 malades ne l'était pas. Les causes de ces échecs sont étudiées.

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RELATIONSHIP OF ISONIAZID TO THE METABOLISM OF MYCOBACTERIA[1]

Catalase and Peroxidase

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INTRODUCTION

Considerable attention has been paid within recent years to the metabolic pathways of mycobacteria. However, in view of the nature of cell structure and the slow growth of tubercle bacilli, not every phase of activity could be well understood. This may be due to the fact, as Edson (1) pointed out, that "being strict aerobes, the mycobacteria transform nutriment into bacterial substance with such a high degree of efficiency that partially oxidized metabolites do not accumulate during the exponential phase of growth." However, with the technique of resting cell preparations, it has been possible to study the mode of utilization of some metabolites. Attention has also been paid recently to the intracellular distribution of enzymes in mycobacteria (2). Although from these researches a great amount of fundamental knowledge has been gained, many aspects remain unsolved.

With the demonstration of the occurrence in tubercle bacilli of absorption bands relative to the cytochromes of heart muscle and of yeast (3), interest was aroused in the role of the cytochrome system in the oxidative metabolism of the mycobacteria. Following the introduction of isoniazid for the treatment of tuberculosis, Middlebrook (4) demonstrated the nature of the relationship between this drug and one of the cytochrome enzymes, catalase. It was observed that bacilli rendered resistant to the drug lost the ability to split hydrogen peroxide to molecular oxygen as there was no detectable catalase present. The development of resistance to isoniazid was also accompanied by a decrease in virulence of the strains studied, and even in total attenuation for the guinea pig (5–7). Further, Fisher (8) demonstrated that hemin is a growth factor for isoniazid-resistant tubercle bacilli and suggested that tubercle bacilli may by-pass isoniazid action by loss of a hemin-synthesizing mechanism.

This relationship of part of the cytochrome enzymes to the development of isoniazid resistance by tubercle bacilli called for attention. Accordingly, some investigations have been carried out at this laboratory in order to understand the nature of the distribution of the cytochrome enzymes in bacilli rendered resistant to various drugs, and in particular to isoniazid. This communication represents the results of studies on two of the cytochrome enzymes, catalase and peroxidase.

[1] From the Medical Section of the Schweizerisches Forschungsinstitut fur Tuberkulose, Davos, Switzerland.

Materials and Methods

The following strains of mycobacteria have been used:

M. tuberculosis, H37Rv[1]
M. tuberculosis, H37Rv-INHR (resistant to 100 γ of isoniazid per ml.)
H37Rv-StrR (resistant to 100 γ of streptomycin per ml.)
H37Rv-PASR (resistant to 100 γ of PAS per ml.)
H37Rv-TB_1R (resistant to 100 γ of amithiozone per ml.)
M. tuberculosis, Ravenel[2]
Ravenel-INHR (resistant to 100 γ of isoniazid per ml.)
M. tuberculosis, BCG[2]
BCG-INHR (resistant to 100 γ of isoniazid per ml.)
M. tuberculosis, H37Ra[2]
M. tuberculosis, H37Rv (NCTC 7416)
M. phlei[2] (saprophyte)
M. smegmatis (saprophyte) (NCTC 8152)
Mycobacterium X-attenuated avian strain (9)
M. tuberculosis, H37Rv (resistant to 0.1, 1.0, and 10.0 γ of isoniazid per ml.)
M. tuberculosis, Ravenel (resistant to 0.1, 1.0, and 10.0 γ of isoniazid per ml.)
M. tuberculosis, BCG (resistant to 0.1, 1.0, and 10.0 γ of isoniazid per ml.)

The mutant strains were produced in this laboratory in order to obtain a closer correlation between parent and mutant, so that differences in enzymatic activity would not be due to mere differences in the strains themselves.

Besides the above, many other strains of tubercle bacilli obtained from patients with different regimens of chemotherapeutic treatment, and having various grades of susceptibility to various drugs (and in particular to isoniazid) were also studied for their catalase and peroxidase activities. These strains bear the numbers "TB—." The drug susceptibilities of all of the strains of mycobacteria were determined on Löwenstein-Jensen medium containing the drugs.

Catalase activity: Qualitative detection was done by the Middlebrook (4) method on Youmans agar or Löwenstein-Jensen medium.

Quantitative estimation was done by the Warburg technique, using 0.2 ml. of 1:100 hydrogen peroxide (30 per cent) as substrate, 2 ml. of 0.2 molar Sørensen phosphate buffer of pH 6.8, and 1 ml. of a bacterial suspension representing approximately 1 mg. of bacteria. The reaction was carried out at 35°C. and 0.2 ml. of 10 per cent sodium hydroxide was kept in the center well. Bacteria were harvested by centrifugation from ten-day Dubos Tween®-albumin medium, washed thrice, and were resuspended in physiologic saline containing 0.025 per cent Tween 80. Suspensions were adjusted photometrically to 85 per cent transmission at 650 mμ, and this represented approximately 1 mg. of dry bacteria per ml.

Peroxidase activity: Peroxidase activity was determined using catechol as acceptor. The bacillary mass from Youmans medium, with or without 10 per cent serum, or Dubos Tween-albumin medium was washed four times with physiologic saline and was suspended in 1 ml. of 0.2 molar acetate buffer of pH 4.0; then 0.2 ml. of 2 per cent aqueous solution of catechol and 0.1 ml. of 1:100 hydrogen peroxide (30 per cent) were added, and incubation was carried out at room temperature (about 20°C.). Strains from the patients were used directly from Löwenstein-Jensen slopes without washing, as it was observed that this medium by itself did not interfere in the enzymatic reaction. Under these conditions, peroxidase-positive strains turn brown within ten minutes and darken and become black after about twelve hours, while peroxidase-negative strains remain unchanged.

[1] Kindly supplied by Dr. William Steenken, Jr., Trudeau Laboratory, Trudeau, New York.

[2] Kindly supplied by Professor P. Hauduroy, University of Lausanne, Lausanne, Switzerland.

TABLE 1

QUANTITATIVE MEASUREMENT OF CATALASE IN MYCOBACTERIA

Strain	Liberation of Oxygen in Microliters per Milligram		
	10 minutes	30 minutes	60 minutes
M. tuberculosis, H37Rv	7.3	19.6	35.9
M. tuberculosis, H37Rv-INHR	0	0	0
M. tuberculosis, H37Rv-StrR	15.2	25.2	30.6
M. tuberculosis, H37Ra	15.2	30.6	35.2
M. tuberculosis, Ravenel	14.4	33.6	32.0
M. tuberculosis, Ravenel-INHR	0	0	0
M. tuberculosis, BCG	6.4	12.8	24.0
M. tuberculosis, BCG-INHR	0	0	0
M. tuberculosis, H37Rv (NCTC)	7.5	22.5	31.5
Mycobacterium X	18.0	48.6	46.8
M. phlei (saprophyte)	93.6	138.6	123.4
M. smegmatis (saprophyte)	32.4	65.1	69.3

RESULTS

The results of quantitative estimations of catalase are presented in table 1. Tubercle bacilli of human and bovine origin susceptible to isoniazid possess almost similar grades of catalase activity, whereas the mutants resistant to isoniazid concentrations of 100 γ per ml. have no catalase. *Mycobacterium* X, probably an attenuated avian strain (9), and especially the saprophytes *M. phlei* and *M. smegmatis* had higher catalase activity.

TABLE 2

PROPERTIES OF PEROXIDASE AND CATALASE OF M. TUBERCULOSIS, H37Rv

		Catalase	Peroxidase
Effect of substrates			
Catechol alone		−	−
Catechol + peroxide		+++	+++
Effect of pH			
0.2 molar Sørensen phosphate, pH 6.8		+++	+
0.2 molar acetate buffer, pH 4.0		++	+++
Effect of temperature			
Exposure to 60°C. for 5 minutes		−	±
Exposure to 95°C. for 2 minutes		−	−
Effect of inhibitors	γ/ml.		
Potassium cyanide	1,000	+	−
	100	+++	−
	10	+++	−
	1	+++	++
Sodium azide	1,000	+	−
	100	+++	−
	10	+++	++
	1	+++	+++

− = No activity; + to +++ = different grades of activity

TABLE 3

RELATIONSHIP OF ISONIAZID SUSCEPTIBILITY TO CATALASE AND PEROXIDASE OF MYCOBACTERIA

Strain	Isoniazid Resistance	Catalase	Peroxidase
	γ per ml.		
M. tuberculosis, H37Rv	Susceptible	+++	+++
M. tuberculosis, H37Rv	100.0	−	−
M. tuberculosis, H37Rv	10.0	−	−
M. tuberculosis, H37Rv	1.0	−	−
M. tuberculosis, H37Rv	0.1	++	−
M. tuberculosis, H37Rv-StrR	Susceptible	+++	+++
M. tuberculosis, H37Rv-PASR	Susceptible	+++	+++
M. tuberculosis, H37Rv-TB_1R	Susceptible	+++	+++
M. tuberculosis, BCG	Susceptible	+++	+++
M. tuberculosis, BCG	100.0	−	−
M. tuberculosis, BCG	10.0	−	−
M. tuberculosis, BCG	1.0	−	−
M. tuberculosis, BCG	0.1	+++	−
M. tuberculosis, Ravenel	Susceptible	+++	+++
M. tuberculosis, Ravenel	100.0	−	−
M. tuberculosis, Ravenel	10.0	−	−
M. tuberculosis, Ravenel	1.0	+	−
M. tuberculosis, Ravenel	0.1	+++	−
M. tuberculosis, H37Rv (NCTC)	Susceptible	+++	+++
M. tuberculosis, TB 565	0.1	++	+
M. tuberculosis, TB 565	1.0	+	−
M. tuberculosis, TB 592	10.0	−	−
M. tuberculosis, TB 592	0.1–10	++	±
M. tuberculosis, TB 282	10	+	−
M. tuberculosis, TB 1171	10	−	−
M. phlei	100*	++++	−
M. smegmatis (NCTC)	100*	++++	−
Mycobacterium X	100*	+++	−

* Naturally resistant to isoniazid.

− = No activity; + to ++++ = different grades of activity

Studies on the nature of peroxidase and catalase of *M. tuberculosis* H37Rv are described in table 2. It may be observed that the conversion of catechol to a brown compound occurred only in the presence of peroxide and that the rate of development of the brown color was higher at pH 4.0 than at pH 6.8. The system was heat labile and highly sensitive to cyanide and a little less sensitive to azide. On the other hand, catalase activity was higher at pH 6.8 and was not as specifically affected by cyanide or azide.

Qualitative determination of catalase and peroxidase activities in relation to isoniazid resistance of mycobacteria are presented in table 3. Tubercle bacilli susceptible to isoniazid exhibited a high degree of peroxidase activity, while the saprophytes and *Mycobacterium* X, which are naturally resistant to isoniazid, did not show the presence of peroxidase under the conditions of the test. Strains

TABLE 4

RELATIONSHIP OF ISONIAZID RESISTANCE TO CATALASE AND PEROXIDASE OF NONACID-FAST ORGANISMS

Organism	Isoniazid Resistance	Catalase	Peroxidase
	γ per ml.		
S. aureus, 7447*	10,000	+++	−
Pseudomonas aeruginosa, 1999*	10,000	+++	−
E. coli, 86*	1,000	++	−
Candida albicans	1,000	++	+
Klebsiella pneumoniae, 7242*	10,000	+	−
S. aureus (penicillin resistant)	10,000	+++	−
Proteus vulgaris, 8309*	10,000	+++	−
Neisseria catarrhalis		+	−
Neisseria meningitides		+	−

* Kindly supplied by the National Collection of Type Cultures, Colindale, England.
− = No activity; + to +++ = different grades of activity.

of tubercle bacilli resistant to streptomycin, para-aminosalicylic acid (PAS), or 4-acetylaminobenzaldehyde thiosemicarbazone (amithiozone), but susceptible to isoniazid, showed the same degree of peroxidase as the parent drug-susceptible strain. Very low levels of isoniazid resistance affected peroxidase to a greater extent than catalase.

Results of experiments on catalase and peroxidase of some nonacid-fast organisms are presented in table 4. It may be noted that only *Candida albicans* possessed some peroxidase, whereas all other strains had very high catalase and no peroxidase.

DISCUSSION

Although much work has been done on the catalase of mycobacteria (10–12), very little is known concerning the nature of peroxidase action. No practical demonstration of the existence of peroxidase has been reported, although some studies with absorption technique (13) indicated the probable existence of the enzyme.

Depending upon the nature of the acceptor, peroxidases have been determined in many different ways. Unlike catalase, peroxidases possess a rather wide range of substrate specificity. In the present study, the orthodiphenol, catechol, was used as the acceptor. Catechol is oxidized to *o*-benzoquinone which condenses to form brown compounds of uncertain composition. The production of the brown color has been taken as the index of enzymatic activity. In order to establish that this reaction is representative of true peroxidase activity, some experiments, presented in table 3, were carried out.

It was found that tubercle bacilli did not convert catechol to the brown compound in the absence of peroxide, and manometric studies revealed that no detectable oxygen was utilized in the presence of catechol or tyrosine by tubercle bacilli. It appears from this that a polyphenol oxidase system is probably absent in tubercle bacilli.

Short-time exposure of the bacteria to heat indicated that the enzyme is heat labile and also that pseudoperoxidase (hemin catalysis) is absent.

Since catechol undergoes autoxidation slowly in the presence of atmospheric oxygen, the possibility of a nonenzymatic reaction involving the active oxygen from catalase was investigated. A preparation of pure crystalline catalase[4] (free from peroxidase and pseudoperoxidase) did not show any activity in browning the catechol, although vigorous liberation of oxygen from hydrogen peroxide was noted. Second, strains such as *M. phlei* and *M. smegmatis* which possess very high catalase also did not show the reaction. Only a preparation of pure peroxidase[5] produced a very rapid browning of catechol in the presence of added hydrogen peroxide. It appeared, therefore, that the browning of catechol was enzymatic and independent of catalase activity.

Cyanide and azide inhibit the production of the brown color by tubercle bacilli specifically as they do with other peroxidases. The enzyme also exhibits a pH optimum near 4.0.

These results are indicative of true peroxidase activity and are consistent with the observations of Theorell (14).

Although the significance of peroxidase in tubercle bacilli is not fully recognized, it is reasonable to suppose that with the slow growth of the species this enzyme may be more involved in the oxidative metabolism than the dehydrogenases which operate with flavine-adenine-nucleotide or diphosphopyridine-nucleotide, or other systems encountered with genera not belonging to the genus *Mycobacterium*.

The present results on catalase of mycobacteria are consistent with the work of others indicating that tubercle bacilli resistant to high concentrations of isoniazid manifest no catalase activity. Although a large number of investigations have been made on the loss of catalase during the development of resistance to isoniazid, the exact significance of this relationship does not appear to have been emphasized. As may be observed from table 1, isoniazid is not active against bacilli (*M. phlei* and *M. smegmatis*) possessing very high catalase or those which have no catalase as a result of resistance development. It appears to the writers, therefore, that this loss in catalase is probably not a direct consequence of resistance, but that it may involve another closely related system which ultimately affects catalase activity.

The results of studies on peroxidase of mycobacteria, presented in table 3, show a better relationship to isoniazid susceptibility. All strains of tubercle bacilli susceptible to isoniazid manifest high peroxidase activity. Bacteria such as *M. phlei*, *M. smegmatis*, and *Mycobacterium* X, which are naturally resistant to isoniazid, do not show any peroxidase. Strains originally susceptible to isoniazid when rendered resistant also lose peroxidase. This observation linking peroxidase, rather than catalase, to isoniazid is more in general agreement, and it appears that

[4] Kindly supplied by C. F. Boehringer & Soehne GMBH, Mannheim, Germany.

[5] Kindly supplied by Professor E. F. Hartree, Molteno Institute of Biology and Parasitology, Cambridge, England.

isoniazid involves peroxidase in some manner. Peroxidase is not diminished in strains resistant to streptomycin, PAS, or amithiozone.

The relative resistance of most of the nonacid-fast organisms to isoniazid prompted a study to see if the resistance is due to lack of peroxidase. The results presented in table 4 indicate that, with the exception of *Candida*, all organisms studied had no peroxidase under the conditions of the experiment. Although *Candida* showed slight peroxidase activity, its resistance to isoniazid is probably due to the rapid removal of hydrogen peroxide by its strong catalase. It must be noted, however, that peroxidase has been detected at pH 4.0 and that catalase is highly inhibited at this pH. As the drug-susceptibility determinations have been carried out at pH 6.8 (at which level catalase has its optimum), peroxidase action is probably masked.

Strains of tubercle bacilli resistant to different concentrations of isoniazid were produced and the enzymatic characteristics of such strains were studied. In this connection, *M. tuberculosis* (H37Rv, Ravenel, and BCG) were made resistant to 0.1, 1.0, and 10.0 γ of isoniazid per ml. individually. It was observed that, while catalase is almost completely lost in strains becoming resistant to isoniazid at 1.0 to 0.1 γ per ml., peroxidase is either diminished at 0.1 γ or completely lost at slightly higher concentrations of isoniazid. Some strains were obtained, besides those presented in this communication, which, on becoming resistant to low concentrations of isoniazid, have lost peroxidase but still retain catalase.

From these studies, it appears that, in view of the very close similarity and relationship between peroxidase and catalase actions, the loss of peroxidase also involves the eventual loss of catalase in tubercle bacilli, although the possibility exists that the two enzymes operate with a single basic protein. The fact that peroxidase is affected to a greater extent than catalase discounts the latter suggestion. If it is a single protein, the loss in enzymatic activity would probably be simultaneous and, further, the saprophytic mycobacteria (*M. phlei* and *M. smegmatis*), which possess very high catalase, exhibit no detectable peroxidase. In view of the very high catalase, it is likely that peroxide is not made available for peroxidase action in these strains. Further work is needed to elucidate the nature of this problem arising out of isoniazid resistance in tubercle bacilli.

In conclusion, it may be stated that the possession of catalase or peroxidase does not make the mycobacteria pathogenic, but that other factors are also involved because the nonvirulent strains BCG and H37Ra have catalase and peroxidase. But it seems fair enough to generalize that possession of peroxidase makes the strain vulnerable to the action of isoniazid in some manner or that, in order to be resistant, the ratio of catalase to peroxidase should be such as to mask peroxidase action. It would also appear that a strain lacking in catalase and peroxidase should be highly susceptible to the peroxide if liberated in tissues.

Summary

Using catechol as acceptor, the existence of peroxidase in mycobacteria has been demonstrated. This enzyme operates at a pH optimum near. 40, is heat labile, sensitive to cyanide and azide, and very distinct from the closely related enzyme,

catalase. A simple technique for the detection of peroxidase in mycobacteria is described.

All mycobacteria resistant to isoniazid, naturally or by mutation, do not possess peroxidase. Peroxidase appears to be essential for the activity of isoniazid not only in mycobacteria but also in the nonacid-fast group of organisms. Isoniazid susceptibility or resistance is more closely related to peroxidase than to catalase. Loss in peroxidase precedes loss in catalase in isoniazid-resistant strains.

Sumario

Relación de la Isoniacida con el Metabolismo de las Micobacterias: Catalasa y Peroxidasa

Usando catecol como aceptador o absorbente, se ha demostrado la existencia de peroxidasa en las micobacterias. Esta encima obra a un pH óptimo próximo a 4.0, es termolábil, sensible al cianuro y a la acida y muy distinto de la encima íntimamente afín, la catalasa. Describese una técnica sencilla para el descubrimiento de la peroxidasa en las micobacterias.

No todas las bacterias resistentes a la isoniacida, ya por naturaleza o por mutación, poseen peroxidasa. La peroxidasa parece ser indispensable para la actividad de la isoniacida no sólo en las micobacterias sino también en el grupo anácidorresistente de microbios. La susceptibilidad o resistencia a la isoniacida está relacionada más íntimamente con la peroxidasa que con la catalasa. En las cepas isoniacidorresistentes, la pérdida de peroxidasa antecede la de catalasa.

Resume

Rapport entre l'isoniazide et le métabolisme des mycobactéries: Catalase et Peroxydase

L'existence de la peroxydase dans les mycobactéries a été démontrée à l'aide de catéchol comme accepteur. Cet enzyme agit à un p^H optimum situé au voisinage de 4, il est labile à la chaleur, sensible au cyanure et à l'azide et très distinct de l'enzyme catalase auquel il est étroitement apparenté. Une simple technique pour la détection de la peroxydase dans les mycobactéries est décrite.

Toutes les mycobactéries résistantes à l'isoniazide, soit naturellement soit par mutation, ne possèdent pas de peroxydase. La peroxydase paraît être essentielle à l'activité de l'isoniazide non seulement chez les mycobactéries mais aussi dans le groupe des germes non acido-résistants. La sensibilité ou la résistance à l'isoniazide est en corrélation plus étroite avec la peroxydase qu'avec la catalase. La perte de peroxydase précède la perte de catalase dans les souches isoniazido-résistantes.

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STUDIES WITH C^{14} LABELED PARA-AMINOSALICYLIC ACID AND ISONIAZID[1,2]

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Introduction

As part of a continuing series of investigations of the metabolism of C^{14} labeled antituberculous drugs (1–5), the writers have been interested in the distribution of C^{14} *p*-aminosalicylic acid (PAS) in both the normal and tuberculous guinea pig. As part of this program, an investigation of C^{14} isoniazid distribution during isoniazid-PAS therapy with unlabeled PAS has been undertaken.

The utilization of the isotope tracer technique for these studies is advantageous from several aspects. The synthesis of C^{14} carboxyl-labeled PAS of known purity in the pharmacology laboratories (6) provided a compound which made possible the direct study of the decarboxylation of PAS *in vivo* both in the guinea pig and in man. The use of C^{14} carboxyl-labeled PAS provided in addition a simple, highly sensitive method for the detection of C^{14} PAS and/or its metabolites in the organs of the normal and tuberculous guinea pig. In addition to the use of PAS labeled in the carboxyl position, the drug was also labeled in 1 position of the benzene ring providing PAS with an isotope atom in a stable location which was utilized for the detection of C^{14} PAS in the caseum of the infected guinea pig at various time intervals. In studying the effect of isoniazid therapy along with PAS, C^{14} isoniazid provided a tracer by which this drug could readily be determined in the presence of PAS.

Materials and Methods

C^{14} carboxyl-labeled p-aminosalicylic acid: C^{14} carboxyl-labeled PAS was synthesized and characterized by a procedure previously described (6).

C^{14} ring-labeled PAS: C^{14} ring-labeled PAS was prepared by a modification of a procedure for the synthesis of PAS devised by McGhie and associates (7), which utilized phenylacetic acid as a starting product. In the synthesis of C^{14} ring-labeled PAS, C^{14} toluene[4] labeled in the 1-carbon of the ring was used as the starting material. The C^{14} toluene was

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[4] Obtained from Tracerlab, Inc.

converted to 1-C^{14} ring-labeled phenylacetic acid. This latter compound was used for the synthesis of 1-C^{14} ring-labeled *p*-nitrosalicylic acid which was then reduced to ring-labeled PAS in a manner analogous to the procedure used in the preparation in this laboratory of C^{14} carboxyl-labeled PAS.

Preparations of solutions of C^{14} NaPAS for injection: C^{14} PAS was solubilized by the use of a slight excess of sodium bicarbonate. Solutions were made up in small amounts and were used for a period of one to two days after preparation. When the solution was refrigerated and protected from light, there was no loss of radioactivity or discoloration. In a number of the experiments on decarboxylation of C^{14} carboxyl-labeled PAS, the sodium salt was prepared by reaction with a cation exchange resin IRC-50 in the sodium form. The soluble salt was then obtained by removal of the resin by filtration and freeze-drying of the resultant solution. Unfortunately, the yields of this preparation tended to vary, and the losses of C^{14} PAS were prohibitive. All solutions of C^{14} NaPAS were prepared with carrier[5] so that 3 ml. of solution contained 200 mg. of PAS acid.

C^{14} isoniazid: The C^{14} isoniazid used for the studies on two-drug therapy was labeled with C^{14} in the carboxyl group and was synthesized by Murray and Langham (8) at the Health Division of the Los Alamos Laboratories, Los Alamos, New Mexico.

Distribution studies of C^{14} PAS in the guinea pig: All guinea pigs, normal and tuberculous, were sacrificed by cervical spine fracture. Blood for assay was obtained by cardiac puncture and was heparinized. The blood was subsequently diluted with distilled water, approximately 1:25; 2 ml. of the final dilution were plated and dried *in vacuo* for C^{14} assay. Urine was removed by syringe from the bladder and the bladder was washed; the urine and washings were then combined, diluted, and plated. The tissues to be analyzed were removed from the animal, blotted free of blood, weighed, and an aliquot was homogenized in glass homogenizers with 85 per cent ethyl alcohol. The homogenates were then filtered by suction and the filtrate was made up to 25 ml. with alcohol and plated on 10 $cm.^2$ copper planchettes which were dried over phosphorous pentoxide *in vacuo* for twelve hours. Use of this extraction procedure avoided losses of radioactivity which occurred if the tissue homogenates were plated directly and dried under a heat lamp. The counting technique is described below. Recoveries for the extraction procedure when known amounts of C^{14} NaPAS were added to the homogenates were 88.4 plus-minus 1.7 per cent.

Distribution studies of C^{14} isoniazid: For determination of C^{14} isoniazid in the tissues of the guinea pig, aliquots of tissue were removed from the animals and homogenized with water. One milliliter fractions of the homogenates were then plated, dried under a heat lamp, and counted. No loss of radioactivity occurred when C^{14} isoniazid was heated in contrast to C^{14} PAS.

Decarboxylation studies: The collection of C^{14} oxygen for the study of the decarboxylation of C^{14} PAS in the guinea pig was conducted in glass metabolism cage systems in which carbon dioxide samples were collected in sodium hydroxide and urine free of fecal material could be obtained (9). In the human studies, the patient was required to breathe into a Douglas bag through a one-way valve and the respiratory air then passed through an alkaline absorption column for collection of the carbon dioxide. $C^{14}O_2$ was determined by neutralization of the sodium hydroxide with 85 per cent phosphoric acid in a modified Van Slyke apparatus (10). The $C^{14}O_2$ was passed into an ionization chamber and assayed on a vibrating reed electrometer[6] (11). Blood and urine radioactivity were determined as described in the distribution studies.

Counting methods: All samples were plated in duplicate and counted in open window gas-flow counters, with efficiencies determined by the counting of a standard planchette. The values obtained were corrected for self-absorption, converted to disintegrations per minute, and are presented as microgram equivalents of C^{14} PAS per gram wet weight of

[5] Unlabeled NaPAS was kindly supplied by Hellwig, Inc. as the dihydrate salt.

[6] Obtained from Applied Physics Corporation, Pasadena, California.

tissue or C^{14} isoniazid per gram dry weight of tissue. A microgram equivalent is defined as the tissue radioactivity equivalent to one microgram of radioactive drug injected. This value does not represent unchanged drug, but rather drug and/or its metabolites.

Observations

Studies with C^{14} carboxyl-labeled NaPAS in normal and infected guinea pigs: Three guinea pigs were infected with a subcutaneous injection of 0.2 mg. wet weight of H37Rv in the inguinal region and were used four to five weeks later. Three normal and three infected guinea pigs were given C^{14} NaPAS (200 mg. of the acid per kg.) intraperitoneally. The organs were removed for C^{14} assay thirty minutes after injection. Preliminary distribution studies revealed that the peak concentrations of C^{14} PAS occurred in most organs at thirty minutes following injection.

In table 1 it may be seen that the highest concentrations of drug were found in the kidney, spleen, and adrenal in the normal animal. Organs such as lung, brain, muscle, and lymph node showed very little variation between normal and tuberculous animals. The differences seen in liver, kidney, spleen, and blood between normal and infected guinea pigs did not prove to be significant by use of the *t* test (12). The lower C^{14} concentrations observed in the adrenals of the infected guinea pig approached significance at the 95 per cent concentration, as did the slight increase in urinary excretion in the infected animals.

TABLE 1

Tissue Distribution of C^{14} PAS Thirty Minutes after Intraperitoneal Injection in the Normal and Infected Guinea Pig at a Dose of 200 Mg. per Kg.

	Normal	Infected
Blood	144	180
Liver	135	153
Lung	119	116
Kidney	481	375
Spleen	238	187
Adrenal	217	154
Muscle	59	55
Brain	11	9
Lymph node	114	113
Urine	13%	19%

Values represent microgram equivalents per gram of wet weight of tissue or milliliter of blood.

TABLE 2

Distribution of C^{14} PAS in Certain Infected Areas Thirty Minutes after Intraperitoneal Injection of 200 Mg. per Kg. of C^{14} PAS

Caseous material	94
Capsule	128
Inguinal node	119

The values represent microgram equivalents of C^{14} PAS per gram wet weight of tissue.

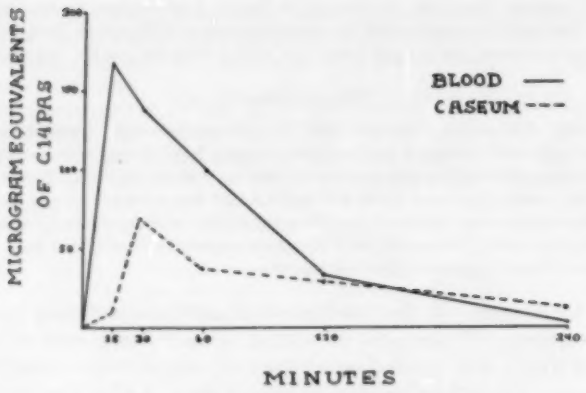

FIG. 1. C^{14} PAS in blood and caseum

Examination of areas of frank infection and caseation (table 2), such as the inguinal lymph nodes, the capsule, and caseous material of a corresponding sinus tract, indicated that in a period of thirty minutes, C^{14} PAS had diffused into these areas in concentrations comparable to lung and liver tissue concentrations. It is apparent that the capsule of the sinus tract does not act as a barrier to the passage of drug into the caseous material.

Time study of C^{14} ring-labeled PAS in the caseum: In addition to the studies described above on the distribution of C^{14} carboxyl-labeled PAS in the infected guinea pig thirty minutes after administration of the drug, a study of C^{14} ring-labeled PAS concentrations in the caseum at various time intervals after intraperitoneal injection was also carried out. For this experiment male and female infected guinea pigs were injected intraperitoneally with C^{14} ring-labeled PAS (200 mg. of the acid per kg.) and then sacrificed at 15, 30, 60, 120, and 240 minutes following the injection and the blood and caseum levels of radioactivity were determined. Each point represents the average of at least 3 animals.

In figure 1 are shown both the blood and caseum concentrations of C^{14} in microgram equivalents of C^{14} ring-labeled PAS. The blood concentration of C^{14} ring-labeled PAS in these animals attained a peak of 170 microgram equivalents within fifteen minutes, while a peak concentration of 70 microgram equivalents per gram wet weight of caseous material was reached in a thirty-minute period. The concentration of C^{14} PAS in the caseum approximately equaled that found in the blood after two hours.

The in vivo decarboxylation of C^{14} PAS in the guinea pig:[7] Ten male guinea pigs weighing from 300 to 400 gm. were fasted for twenty-four hours prior to the administration of C^{14} NaPAS (200 mg. of the acid per kg.), either orally, by stomach tube, or intraperitoneally. All 10 animals were lightly anesthetized with Vinethene® to facilitate the passing of a

[7] This work was presented in abstract form in J. Pharm. & Exper. Therap., 1956, *116*, 28.

stomach tube. $C^{14}O_2$ was collected from the animals at one, eight, and twenty-four hours, and urine was obtained for twenty-four- and forty-eight-hour periods.

In table 3 are presented the data on the percentage of the C^{14} dose which appeared in the respiratory air after oral and intraperitoneal administration of C^{14} NaPAS. A greater amount of $C^{14}O_2$ was produced after oral administration. Urinary excretion values are presented in table 4. The range of excretion values after oral administration is 49 to 92 per cent of the administered dose. This is in general agreement with the work of McCloskey and associates (13), who found that the urinary excretion of unlabeled PAS in the guinea pig was 51 to 79 per cent of the administered dose in a twenty-hour period. The urinary excretion after intraperitoneal injection was not significantly different from that following oral dosage.

In order to obtain more information concerning the site of decarboxylation after oral administration of C^{14} NaPAS, a series of animals which had been operated upon was studied.

Five guinea pigs were anesthetized with Vinethene and a double ligature was passed at the junction of the stomach and duodenum, C^{14} NaPAS being administered orally. Five sham animals operated upon served as controls. In order to eliminate the possibility that the $C^{14}O_2$ appearing in the respiratory tract was not excreted via the esophagus and mouth, but was actually respiratory $C^{14}O_2$, the stomachs of 2 animals were ligated at the pylorus and cardia and the drug was injected directly into the stomach. In 2 of the animals, ligatures were placed at the pylorus and the drug was injected into the duodenum. All ligated animals were examined post mortem or at the termination of the experiment, and the ligatures were found to be intact. The stomachs of these animals were greatly dilated. As the ligated animals died, frequently before a twenty-four-hour period had elapsed, the $C^{14}O_2$ values for the animals operated upon are given for only the first- and eight-hour periods. Urinary C^{14} was determined for the period during which the animals survived, usually between eighteen and twenty-four hours.

TABLE 3

EXCRETION OF $C^{14}O_2$ FROM GUINEA PIGS ADMINISTERED C^{14} CARBOXYL-LABELED NaPAS

Route of Administration	1 Hour	8 Hours	24 Hours	24-Hour Total
Oral	1.7	2.0	0.4	4.1
Intraperitoneal	0.1	0.2	0.1	0.4

Data expressed as per cent of the dose.

TABLE 4

URINARY EXCRETION OF C^{14} FROM GUINEA PIGS ADMINISTERED C^{14} CARBOXYL-LABELED NaPAS

Route of Administration	24 Hours	48 Hours	48-Hour Total
Oral	72.9	6.0	78.9
Intraperitoneal	80.0	6.8	86.8

Data expressed as per cent of the dose.

TABLE 5

EXCRETION OF C^{14} FROM GUINEA PIGS OPERATED UPON, WHO WERE ADMINISTERED C^{14} CARBOXYL-LABELED NaPAS

Animal	C^{14} Oxygen Excretion			Urinary Excretion During Survival Period
	1 Hour	8 Hours	8-Hour Total	
Surgery: pylorus ligated. Route: oral	2.0	9.1	11.1	64.9
Surgery: sham operated. Route: oral	1.3	6.2	7.5	64.2
Surgery: pylorus and cardia ligated. Route: stomach injection	2.3	8.4	10.7	57.1
Surgery: pylorus ligated. Route: duodenal injection	0.0	0.1	0.1	79.2

Data expressed as per cent of the dose.

The ligation of the stomach prior to oral dosage led to a pronounced increase in the $C^{14}O_2$ production (table 5). In the case of the sham animals, there was also an increase of $C^{14}O_2$ in the respiratory air above that seen on simple oral administration. Nevertheless, the difference between the ligated and sham animals was significant. Double ligation of the pylorus and cardia of the stomach, with injection of C^{14} NaPAS directly into the stomach, led to values of 10 per cent in eight hours, and the difference in the means of these and the pyloric ligated animals which received oral doses was not significant. Direct injection into the duodenum, however, led to only trace amounts of C^{14} in the respiratory carbon dioxide. The urinary values for C^{14} excretion indicated that the drug was more rapidly excreted when it was injected directly into the duodenum.

Studies with C^{14} carboxyl-labeled PAS in the human: The urinary and fecal excretion, blood concentrations, and production of C^{14} oxygen after the oral administration of a dose of C^{14} NaPAS was determined in a human volunteer undergoing therapy for tuberculosis. The patient, W.R., a 26-year-old male was admitted to the hospital with advanced pulmonary tuberculosis. Under therapy with isoniazid-PAS there was almost complete resolution of the tuberculous lesion. At the time of this experiment, the patient had sputum negative for tubercle bacilli.

Three and a half grams of NaPAS containing 17 microcuries of C^{14} PAS were administered as a regular dose of PAS. Blood and urine samples were obtained at various time intervals for C^{14} assay. Immediately following the administration of the oral dose of the drug, a five-minute sample of the expired air was collected. Thereafter, air samples were taken simultaneously with blood and urine up to twelve hours after the dose was given.

Blood determinations showed that the peak concentrations of 10.1 mg. of PAS per 100 ml. was reached in thirty minutes. By twelve hours the concentration had fallen to 1.2 mg. per 100 ml. Urinary excretion was rapid, 50 per cent of the dose appearing in two hours and 75 per cent being excreted in four hours. The twenty-four-hour fecal sample contained 4.4 per cent of the dose, while the twenty-four- to forty-eight-hour collection contained 0.5 per cent. Although there was a detectable amount of radioactivity found in the respiratory air, the total $C^{14}O_2$ excreted amounted to only 1.0 to 1.5 per cent of the total dose. The peak of this respiratory excretion occurred in twelve minutes. The total C^{14} re-

TABLE 6

DISTRIBUTION OF C^{14} ISONIAZID AFTER A SINGLE INJECTION OF C^{14} ISONIAZID (10 MG. PER KG) ALONE OR IN COMBINATION WITH NaPAS (200 MG. PER KG.)

	One Hour		Two Hours	
	C^{14} Isoniazid	C^{14} Isoniazid and NaPAS	C^{14} Isoniazid	C^{14} Isoniazid and NaPAS
Blood	7.0	5.3	3.8	4.9
Liver	29.7	26.7	18.9	22.4
Spleen	30.6	26.1	16.6	23.5
Lung	37.2	28.0	19.6	24.9
Gut	126.0	184.7	157.6	127.8
Urine	13.3%	7.3%	26.6%	30.5%

Values represent microgram equivalents per gram of wet weight of tissue or milliliter of blood.

covery from this patient, including urine, feces, and $C^{14}O_2$, amounted to 92 per cent of the administered dose in a forty-eight-hour period.

Distribution of C^{14} isoniazid during therapy with isoniazid and NaPAS: For the study of the distribution of C^{14} isoniazid during combined therapy with PAS, male guinea pigs weighing from 300 to 600 gm. were injected intraperitoneally with either C^{14} isoniazid alone in a dose of 10 mg. per kg. or C^{14} isoniazid plus a simultaneous injection of 200 mg. of PAS per kg. as the sodium salt. These animals were sacrificed at one or two hours after intraperitoneal injection and the C^{14} content of the tissues was determined.

In table 6 are shown the results of this study. Each value in this table represents a minimum of 6 animals. The differences seen in the data at either one or two hours after injection between animals given C^{14} isoniazid alone or along with NaPAS did not prove to be statistically significant. There was considerable individual variation in the distribution of C^{14} isoniazid from animal to animal, but these differences were not found to be related to the administration of PAS.

A significant finding of this investigation was the C^{14} isoniazid concentrations in the intestinal tract of the experimental animals. The values given in table 6 for the gut represent activity per gram dry weight of the total gastrointestinal tract and its contents. An estimation of the total amount of C^{14} isoniazid in the gastrointestinal tract gives a value of approximately 20 to 40 per cent of a given dose of the drug. More important was the fact that, although there was considerable variability in tissue concentrations of C^{14} isoniazid, it could be correlated with the amount in the gastrointestinal tract; that is, in animals in which the tissue concentrations were low, it could be accounted for by an increase in the intestinal content of C^{14} isoniazid.

DISCUSSION

Although there have been a number of studies on the tissue distribution of PAS in various animal species (14–16), the writers are not aware of any report on the distribution of this compound in the tuberculous animal. Previous work in

this laboratory (4) on the distribution of C^{14} isoniazid in the normal and infected guinea pig indicates that the presence of the caseous lesion in a tissue has an effect on the retention of this important antituberculous agent. It was observed that C^{14} concentrations could be correlated with the amount of caseation in the organ and that infected tissues such as spleen and lung tended to accumulate the drug. The most striking differences seen between normal and infected animals were the higher tissue concentrations found in the infected lymph nodes. It was concluded that caseous material had an affinity for the drug and thus grossly infected organs retained larger quantities of C^{14} isoniazid.

In the present studies on the distribution of C^{14} PAS in normal and infected animals, it was found that the presence of caseation caused no change in the distribution of C^{14} PAS in areas of caseation, and a time study of C^{14} ring-labeled PAS in the caseum showed that C^{14} PAS and/or its metabolites diffused readily into the caseous material, reaching peak concentrations within one-half hour. The fact that the presence of the tuberculous process in the tissues has no effect on the distribution of C^{14} PAS and the diffusion of C^{14} ring-labeled PAS out of the caseum seems to indicate that the caseum does not have an affinity for C^{14} PAS and the drug would therefore not be retained in infected organs in contrast to C^{14} isoniazid.

With the synthesis and characterization of C^{14} carboxyl-labeled PAS, it became possible to study the decarboxylation of PAS *in vivo* directly. Studies by Venkataraman and co-workers (17) indicated that PAS was not decarboxylated by incubation with either rabbit or rat stomach or intestinal content homogenates. It was further concluded by these workers that PAS is not decarboxylated to an appreciable extent *in vivo*. This conclusion is based on the absence of metabolites of *m*-aminophenol and the comparison between urinary excretion values when estimated by means of either the ferric chloride reagent, which requires both the carboxyl and hydroxyl groups of PAS, and Erlich reagent, requiring only the amino group for color development. Bray, Thorpe, and Wood (18), in studying the metabolism of aminohydroxybenzoic acids, found that chromatography of acid extracts of urine from rabbits administered PAS showed the presence of *m*-aminophenol and therefore concluded that PAS did undergo decarboxylation to a small extent *in vivo*. Way (19) found *m*-aminophenol as one of five trace products in human urine.

In the present studies on the decarboxylation of C^{14} PAS in the guinea pig, it vas shown that when C^{14} NaPAS was administered orally, 4 per cent of the administered dose appeared as $C^{14}O_2$. The fact that intraperitoneal injection led to the decarboxylation of only 0.4 per cent of the dose and, in addition, the increased decarboxylation produced by ligation of the pylorus prior to an oral dose of the drug indicated that the major site of the decarboxylation is the stomach. The fact that only trace amounts of $C^{14}O_2$ were produced after C^{14} PAS was injected into the duodenum after ligation of the pylorus showed that the reaction was not of a metabolic nature but, rather, was due to the contact of C^{14} PAS with acid in the stomach. The finding that there was no difference between oral administration after pyloric ligation and injection of the drug into the stomach with the cardia also ligated showed that $C^{14}O_2$ was being absorbed

from the stomach and was not simply being excreted via the esophagus. Absorption of C^{14} NaPAS from the stomach was indicated by its excretion in the urine of all of the ligated animals, although the urinary excretion was somewhat greater when the injection was directly into the duodenum.

In the human subject, it was again apparent that C^{14} NaPAS is decarboxylated when administered orally; however, the amount of drug appearing as $C^{14}O_2$ was only 1.0 to 1.5 per cent of the dose. Determination of C^{14} PAS concentrations in the blood were in reasonable agreement with those of Wilson and Armstrong (20) although these workers used a higher dosage of PAS. C^{14} NaPAS was found to be rapidly excreted in the urine of the human, and radioactivity was also excreted in the feces.

Due to the fact that the main value of PAS in the chemotherapy of tuberculosis lies in its use in association with isoniazid or streptomycin, a series of studies has been started dealing with the distribution and metabolism of C^{14} isoniazid during isoniazid-PAS therapy. With regard to the possible effects of PAS on other chemotherapeutic agents, Short has shown (21) in a number of species that PAS, when given with a sulfone (sulphetrone), can produce a rise in the sulfone concentrations in the blood. In the mouse there is, in addition, a depression of the tissue concentrations of the sulfone with simultaneous administration of PAS, presumably on the basis of competition between PAS and the sulfone for protein receptors, with the balance in favor of PAS. In addition to those observations by Short, the interest of the writers in this phase of multiple-drug chemotherapy was stimulated by the work of Johnson (22) and Johnson and Corte (23) who reported that there was a competitive inhibition of isoniazid acetylation by PAS and other amino, amido, and hydrazino compounds in the pigeon liver extract. In addition, he found in the rabbit that both PAS and sulfanilamide, when administered with isoniazid, raised the plasma concentrations of free isoniazid. In man, it has been shown by Mandel and associates (24) that PAS raises the isoniazid concentrations as determined by microbiologic assay of the serum.

It is apparent from the present studies in the guinea pig that at the one- and two-hour periods following the administration of the labeled drug there is no effect of PAS on the blood or tissue concentrations of C^{14} isoniazid and/or its metabolites. It should be pointed out that in these studies at present no distinction can be made between isoniazid and its metabolites, and the results are therefore not necessarily at variance with the previous reports mentioned above. It was also found in the present studies that there was a great deal of variation in the blood and tissue drug concentrations of guinea pigs given C^{14} isoniazid which is apparently unrelated to the associated administration of PAS but appears to be associated with the amounts of drug found in the gastrointestinal tract. Studies to clarify the role of PAS and the gastrointestinal tract in C^{14} isoniazid metabolism are now under way.

Summary

The presence of tuberculous caseation in infected tissues had no effect on the distribution of C^{14} carboxyl-labeled PAS. Both carboxyl and ring-labeled PAS

were found to penetrate readily the caseous material and within thirty minutes reached concentrations comparable with those in the liver and lung. No evidence was found to indicate that caseous material has an affinity for C^{14} PAS as is the case with C^{14} isoniazid.

Studies on the decarboxylation of C^{14} carboxyl-labeled PAS in the guinea pig show that C^{14} PAS is decarboxylated to the extent of 4.0 per cent in twenty-four hours when given orally and to 0.4 per cent when administered intraperitoneally. The major site of this decarboxylation is the stomach, presumably due to the acid condition of this organ. An experiment in a human volunteer showed also that C^{14} PAS is decarboxylated in the human, but only to the extent of 1.0 to 1.5 per cent of an orally administered dose.

Initial studies on multiple-drug therapy with C^{14} isoniazid and unlabeled PAS indicated that the distribution of C^{14} isoniazid and/or its metabolites at one and two hours following administration was not affected by the simultaneous administration of PAS. It has been noted in these studies that there is considerable variation in the blood and in the tissue concentrations of C^{14} isoniazid which is apparently unrelated to PAS administration, but which may be correlated with the amount of radioactivity found in the gastrointestinal tract.

Sumario

Estudios con el PAS y la Isoniacida Rotulados con C^{14}

La presencia del proceso caseoso en los tejidos infectados no ejerce efecto alguno sobre la distribución del PAS rotulado con carboxilo C^{14}. Según se observó, tanto el PAS rotulado con carboxilo como el ánulo-rotulado penetran sin dificultad en las materias caseosas, alcanzando en treinta minutos concentraciones comparables a las presentes en el hígado y el pulmón. No se encontraron datos indicativos de que las materias caseosas muestren afinidad hacia el PAS-C^{14} como sucede con la isoniacida-C^{14}.

Estudios relativos a la decarboxilación del PAS rotulado con carboxilo O^{14} en el cobayo muestran que el PAS-C^{14} es decarboxilado en una proporción de 4.0 por ciento en veinticuatro horas cuando se administra oralmente y de 0.4 por ciento cuando se administra intraperitonealmente. El principal sitio de esta decarboxilación es el estómago, presuntamente debido a la acidez de este órgano. Un experimento en un voluntario humano demostró además que el PAS-C^{14} es decarboxilado en el hombre, pero sólo en la proporción de 1.0 a 1.5 por ciento de una dosis administrada oralmente.

Estudios iniciales sobre la terapéutica combinada con isoniacida-C^{14} y PAS no rotulado indicaron que la distribución de la isoniacida-C^{14} y/o de sus metabolitos a la hora y las dos horas de su administración no es afectada por la administración simultánea de PAS. En estos estudios, se ha notado que existe considerable variación de las concentraciones de isoniacida-C^{14}, en la sangre y el tejido lo cual no está aparentemente relacionado con la administración de PAS, pero puede correlacionarse con la cantidad de radioactividad descubierta en el tubo gastrointestinal.

Resume

Etudes effectuées avec le PAS et l'isoniazide marqués avec C^{14}

La présence d'un processus caséeux dans les tissue infectés est sans effet sur la distribution du PAS marqué par le carboxyle C^{14}.

Il a été observé que le PAS marqué dans la position carboxyle ainsi que dans la position 1 du noyau benzenique pénétrait facilement dans la substance caséeuse, atteignant des concentrations comparables à celles du foie et du poumon en 30 minutes. Il n'a été

decouvert aucun signe indiquant que la matière caséeuse avait une affinité pour le PAS C^{14} comme c'est le cas pour l'isoniazide C^{14}.

Les études sur la décarboxylation du PAS marqué par le carboxyle C^{14} chez le cobaye montrent que le PAS C^{14} est décarboxylé jusqu'à 4% en vingt-quatre heures, quand il est administré oralement, et jusqu'à 0,4% quand il est injecté dans le péritoine. Le siège principal de cette décarboxylation est l'estomac, ce qui est probablement dû au milieu acide présent dans cet organe. L'expérimentation chez un volontaire humain montrait également que le PAS C^{14} est décarboxylé chez l'homme, mais seulement jusqu'à 1 àl, 5% pour la dose administrée oralement. Une étude initiale sur le traitement combiné avec l'isoniazide C^{14} et le PAS non marqué indique que la répartition de l'isoniazide C^{14} et de ses métabolites ou de ces différentes substances, une et deux heures après l'administration n'est pas affectée par l'administration simultanée du PAS. Dans ces études, il a été observé qu'il existe une variation considérable dans les concentrations de l'isoniazide C14 dans le sang et les tissus qui semble être sans relation avec l'administration du PAS mais qui peut être en rapport avec la valeur de la radioactivité présente dans la voie gastro-intestinale.

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EFFECT OF EXPERIMENTAL TUBERCULOSIS ON THE LIPIDS OF RABBIT TISSUES[1]

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INTRODUCTION

The lipid composition of tuberculous tissues has been the subject of rather extensive investigation (1). Unfortunately, most of the studies have contributed little to our understanding of the tuberculous process because no comparisons were made with normal tissues. Moreover, much of the more recent work, such as that of Grogg and Pearse (2), has relied on histochemical methods which do not lend themselves readily to precise qualitative and quantitative comparisons.

Recent studies demonstrating that resistance to tuberculous infection can be increased in experimental animals by dietary administration of certain fats (3–5), possibly by alteration of the biochemical environment in the host tissue, have renewed interest in the effect of the disease process itself on lipid composition of the tissues. In the work to be described, rabbits seemed to be the animal of choice for two reasons. They would allow comparison of the effects produced by tubercle bacilli of human origin, to which the animal is rather resistant, with those produced by organisms of bovine origin, to which the rabbit is extremely susceptible. Moreover, from the standpoint of pathology, the host response to tuberculous infection in the rabbit resembles that which occurs in man (6, 7).

MATERIALS AND METHODS

Strains of tubercle bacilli: The source and maintenance of the strains of human (H37Rv) and bovine (Ravenel) tubercle bacilli used in these studies have been described elsewhere (8).

Infection of animals and collection of tissues: A portion of the pellicle from fourteen-day-old cultures grown on a modified Proskauer and Beck liquid medium (9) was ground and standardized to contain 0.1 mg. per ml. according to the technique described by Youmans and Karlson (9). Twenty-four male albino rabbits weighing approximately 2,000 gm. were divided into three groups. Eight were inoculated intravenously with 1 ml. (0.1 mg.) of the H37Rv suspension, 8 were inoculated with 1 ml. (0.1 mg.) of the Ravenel suspension, and 8 served as uninfected controls. At the same time, further dilutions of each bacterial suspension were prepared and inoculated onto solid egg media in order to have confirmatory evidence that each of the two bacterial suspensions contained approximately the same number of viable units. Infected and control rabbits were maintained on the same diet (commercial pellets and cabbage) throughout the experimental period.

On the seventeenth day of infection all of the animals were fasted for eighteen hours and killed by occipital trauma. Total lung and approximately two liver lobes were immediately removed from each rabbit, blotted with filter paper, placed in a tared Petri dish, and weighed. Each tissue sample was then homogenized in six volumes of ethanol in a Servall Omni Mixer and stored at −20°C.

Lipid extraction and fractionation: Sufficient ethyl ether was added to each alcoholic

[1] From the Tuberculosis Research Laboratory, Veterans Administration Hospital, Washington, D. C.

homogenate to make a 3:1 alcohol-ether mixture. The "minces" were extracted for one hour on a 70°C. water bath and the alcohol-ether was decanted through filter paper. The tissue residue was re-extracted twice with fresh alcohol-ether, transferred to the filter paper, washed thoroughly with ethyl ether, and dried to constant weight in a 70°C. oven. The ethyl ether washings were added to the combined alcohol-ether extracts and the solvent mixture was concentrated to 5 ml. under nitrogen on a 65°C. water bath. The lipids were thoroughly extracted with petroleum ether. The petroleum ether extracts were evaporated to 5 ml. under nitrogen and the phospholipids were precipitated by adding 25 ml. of acetone and 10 drops of a 5 per cent alcoholic solution of magnesium chloride. The mixture was centrifuged and the acetone supernate was decanted and saved. The precipitated phospholipids were washed twice with fresh acetone and, after centrifugation, the acetone washes were combined with the original acetone supernate.

The phospholipids were transferred to a saponification flask equipped with an air condenser and refluxed in a boiling water bath for four hours with 50 ml. of 2 M alcoholic potassium hydroxide. The contents of the flask were cooled, 100 ml. of distilled water were added, and unsaponifiable material was removed with petroleum ether. The aqueous alcohol layer was acidified with 5 N hydrochloric acid. The *phospholipid fatty acids* were extracted with ethyl ether, washed with distilled water, and stored at −20°C. under nitrogen.

The acetone was removed from the nonphospholipid (acetone-soluble) fraction under a stream of nitrogen and the residue was taken up in ethyl ether. Free fatty acids were isolated from this material by shaking it with two 25 ml. portions of 2 per cent potassium hydroxide. The ether layer was saved and the pooled alkaline layers were extracted three times with ethyl ether. These extracts were pooled with the original ether layer. The alkaline layer was acidified with 5 N hydrochloric acid. The *free fatty acids* were extracted with ethyl ether, washed with distilled water, and stored at −20°C. under nitrogen.

The ether fraction, containing glycerides, sterols, and sterol esters, was transferred to a saponification flask and the solvent was removed under a stream of nitrogen. The residue was refluxed with potassium hydroxide as described above for phospholipids, and unsaponifiable material was removed with petroleum ether. After acidification with 5 N hydrochloric acid the *glyceride fatty acids*[2] were extracted with ethyl ether, washed with distilled water, and stored at −20°C. under nitrogen.

Fatty acid determinations: Yields of the three fatty acid fractions were determined by a modification (10) of the method described by Bragdon (11). The extremely small yield of free fatty acids precluded further studies; however the iodine number (12) and saponification equivalent (13) of the phospholipid and glyceride fatty acids were determined on duplicate aliquots of each fatty acid sample. The iodine number and saponification equivalent of each sample were used to calculate the number of double bonds per molecule.

Statistical analysis: The significance of the differences from the normal means was calculated by the *t* test (14).

Results

Organ weights and per cent dry, fat-free tissue residue: In order to have a basis for calculating the yields of fatty acids, the fresh weight and the amount of fat-free residue were recorded for each tissue sample. Since this information was available, it seemed to be of interest to determine whether significant differences could be observed between normal and infected animals. These results are presented in table 1.

The mean fresh lung weight of the 8 control rabbits was 9.9 gm. A significant

[2] For the sake of convenience this fraction will hereafter be designated as "glyceride fatty acids." It should be pointed out, however, that the material could conceivably contain small amounts of fatty acids derived from sterol esters.

TABLE 1

EFFECT OF EXPERIMENTAL TUBERCULOSIS IN RABBITS ON ORGAN WEIGHTS AND PERCENTAGE OF DRY, FAT-FREE RESIDUE

		Control	H37Rv	Ravenel
Lung	Fresh weight (gm.)	9.9	17.4	27.0
	t value		3.00*	6.39*
	Dry, fat-free tissue residue (gm.)	1.4	2.5	4.0
	t value		2.82*	5.95*
	Per cent dry, fat-free tissue residue	14.5	14.3	14.9
	t value		0.30	0.81
Liver†	Per cent dry, fat-free tissue residue	21.0	19.6	17.5
	t value		3.21*	7.79*

* Statistically significant at 0.02 probability level.

† Comparisons could not be made between the infected and control animals using the fresh weights and residue weights of the liver samples since they did not represent the total liver but only a portion thereof.

TABLE 2

EFFECT OF EXPERIMENTAL TUBERCULOSIS IN RABBITS ON THE FREE FATTY ACID CONTENT OF LUNG AND LIVER

		Control	H37Rv	Ravenel
Lung	Mg./gm. fresh tissue	0.12	0.07	0.06
	t value		3.97*	5.22*
	Mg./gm. tissue residue	0.8	0.4	0.4
	t value		4.39*	4.92*
Liver	Mg./gm. fresh tissue	0.08	0.08	0.07
	t value		0.00	2.49
	Mg./gm. tissue residue	0.4	0.4	0.4
	t value		0.00	0.00

* Statistically significant at 0.02 probability level.

increase was observed in animals infected with the H37Rv strain. An even greater increase occurred in the Ravenel-infected rabbits, in which the mean lung weight (27.0 gm.) was significantly greater than that of both the control and the H37Rv-infected animals. The mean values for the amount of dry, fat-free lung residue followed the same trend as that observed with the fresh weights. The infected lungs did not differ from the normal lungs in their per cent of dry, fat-free tissue residue.

Comparisons could not be made between the infected and control animals, using the fresh weights and fat-free residue weights of the liver samples, since they did not represent the total liver but only a portion thereof. The values for the percentage of fat-free residue, however, revealed an interesting difference from the results obtained with the lung. A significant decrease occurred in the livers of animals infected with the H37Rv strain. An even greater decrease was

evident in the Ravenel-infected rabbits in which the mean percentage (17.5) was significantly lower than the values for fat-free residue in both the control and the H37Rv-infected animals.

Free fatty acids: The lungs of both groups of infected animals had a significantly lower content of free fatty acids than did lungs of the control animals (table 2). There was no apparent difference between H37Rv-infected and Ravenel-infected rabbits. The disease did not result in any alteration in the free fatty acid content of the liver.

Glyceride fatty acids: Experimental tuberculosis resulted in approximately a 60 per cent (H37Rv) to 70 per cent (Ravenel) reduction in glyceride fatty acids in the lungs (table 3). The difference between the H37Rv and Ravenel groups, however, was not significant. The number of double bonds per molecule, iodine number, and saponification equivalent of the lung glyceride fatty acids were not altered by infection.

Tuberculous infection did not cause any change in the glyceride fatty acid content of the liver, nor did it affect the number of double bonds per molecule or iodine number. The saponification number of the liver glyceride fatty acids, however, was decreased in rabbits infected with the H37Rv strain whereas no such change occurred in those infected with the Ravenel strain.

Phospholipid fatty acids: Tuberculous infection did not result in any change in

TABLE 3

EFFECT OF EXPERIMENTAL TUBERCULOSIS IN RABBITS ON THE GLYCERIDE FATTY ACIDS OF LUNG AND LIVER

		Control	H37Rv	Ravenel
Lung	Mg./gm. fresh tissue	15.8	6.9	4.0
	t value		3.70*	7.02*
	Mg./gm. tissue residue	108.1	48.0	27.1
	t value		3.75*	7.45*
	Saponification equivalent	221	197	232
	t value		1.35	0.58
	Iodine number	80	79	88
	t value		0.15	1.63
	Number double bonds/molecule	0.69	0.61	0.81
	t value		1.05	1.59
Liver	Mg./gm. fresh tissue	13.8	13.6	11.5
	t value		0.08	0.99
	Mg./gm. tissue residue	66.1	68.4	66.2
	t value		0.16	0.01
	Saponification equivalent	277	246	267
	t value		2.89*	0.71
	Iodine number	49	66	70
	t value		1.94	1.81
	Number double bonds/molecule	0.52	0.64	0.74
	t value		1.42	1.59

* Statistically significant at 0.02 probability level.

TABLE 4

EFFECT OF EXPERIMENTAL TUBERCULOSIS IN RABBITS ON THE PHOSPHOLIPID FATTY ACIDS OF LUNG AND LIVER

		Control	H37Rv	Ravenel
Lung	Mg./gm. fresh tissue	12.3	14.3	13.9
	t value		1.52	0.96
	Mg./gm. tissue residue	85.2	100.7	93.6
	t value		1.27	0.67
	Saponification equivalent	300	294	275
	t value		0.59	2.12
	Iodine number	88	92	110
	t value		0.76	5.83*
	Number double bonds/molecule	1.04	1.06	1.19
	t value		0.33	2.45
Liver	Mg./gm. fresh tissue	27.6	25.6	20.8
	t value		1.57	5.56*
	Mg./gm. tissue residue	131.9	130.4	119.1
	t value		0.27	2.10
	Saponification equivalent	278	280	266
	t value		0.15	0.95
	Iodine number	107	105	108
	t value		0.47	0.12
	Number double bonds/molecule	1.17	1.15	1.13
	t value		0.34	0.41

* Statistically significant at 0.02 probability level.

the yield, number of double bonds per molecule, or the saponification equivalent of phospholipid fatty acids isolated from the lung (table 4). In rabbits infected with the Ravenel strain, however, the iodine number of these acids was significantly increased.

Calculated on the basis of mg. per gm. of fresh tissue, the yield of phospholipid fatty acids was decreased in the livers of rabbits infected with the Ravenel, but not the H37Rv, strain. This decrease loses its statistical significance when the yields are based on the fat-free residue. This discrepancy is probably related to the fact that infection caused a reduction in the percentage of residue in the liver (table 1). The number of double bonds per molecule, iodine number, and saponification equivalent of the phospholipid fatty acids isolated from liver were not altered by infection.

DISCUSSION

The lipids of comparatively few species of rodent have been studied in any great detail. As far as the rabbit is concerned, information available to date relates mostly to the component fatty acids of interscapular (15) and perinephric (15, 16) fats. Miura (17) has reported an analysis of the fat and phospholipid composition of rabbit liver, muscle, and kidney. However, the normal values determined in the present study have, to the writers' knowledge, not been re-

ported previously. The value for the saponification equivalent of glyceride fatty acids from rabbit lung, 221, is of particular interest since it would suggest the presence of relatively short-chain fatty acids in larger amounts than are ordinarily encountered in animal fats. The perinephric fat of the rabbit, for example, has been shown (16) to consist of 95.5 per cent of fatty acids of the C_{16} to C_{18} series and only 4.5 per cent of myristic acid (saponification equivalent, 228). In the present study, the saponification equivalent of the fatty acids from liver glycerides (table 3) and from lung and liver phospholipids (table 4) are also consistent with the presence of predominantly long-chain fatty acids.

Although rabbits are considered to be resistant to tubercle bacilli of human origin, it seems fairly well established that destruction of bacilli does not take place before some preliminary multiplication (18). The infection period (seventeen days) used in the present study was chosen purposely as representing that point at which multiplication of tubercle bacilli of either human or bovine origin would be occurring. Moreover, it was believed that a rather short infection period would exclude biochemical alterations which might result, not from the infection per se, but from the poor nutritional state of the infected animals. It is unlikely that malnutrition was responsible for the effects observed in the present study since fat infiltration in the liver, readily observed in rabbits in starvation (19), was not apparent.

It has been shown recently (20) that in the lung and liver of normal rabbits the different lobes vary markedly in their lipid content. For this reason the entire lung or, in the case of liver, the same two lobes were removed from each animal for lipid analysis. The increase in weight of lungs of infected rabbits finds an interesting correlation in studies of experimental tuberculosis in mice (21) which have shown that the enlargement and increase in weight of lungs are proportional to the intensity of the lesions. It is interesting that the mean lung weight of the H37Rv-infected rabbits was almost twice, and that of the Ravenel-infected animals nearly three times, the weight of lungs from the controls. Examination of the values for the percentage of dry, fat-free tissue residue reveals a difference between lung and liver. In the lung there was no difference between the normal and the infected animals, whereas in the liver there was a significant decrease, more so for the Ravenel-infected than for the H37Rv-infected rabbits. It is possible that these differences might be due to edema rather than to any actual alteration in the absolute concentration of some nonlipid tissue component. Water comprised approximately 73.6, 75.2, and 78.4 per cent of the liver of control, H37Rv-infected, and Ravenel-infected animals, respectively.

The significance of the small amounts of free fatty acids in animal tissues is not known. Early in the course of his work, Bloor (22) suggested that, on the basis of their iodine number, the free fatty acids of beef heart were probably derived from neutral fat. It might be suggested that fatty acids do not exist free in living tissues and their presence is due simply to autolytic decomposition of cell lipids during the analysis. Using techniques designed to minimize autolysis, Fairbairn (23) found that the free fatty acid content of whole mice was very small (2.2 mg. per gm. dry, fat-free tissue). In studies of blood plasma (24), the term "unesteri-

fied fatty acid" is used in preference to "free fatty acid" due to evidence that almost all of the fatty acids in plasma are firmly, though reversibly, bound to serum albumin. The same situation could conceivably apply in the tissues and, in fact, the hypothesis has been offered that the unesterified fatty acid is the blood lipid fraction concerned with the supply of fats to tissues for oxidative metabolism (24). However, at the present time very little is known concerning the source, character, and purpose of the free fatty acids which have been found in trace amounts in various animal tissues. Suffice it to say that in the present study the quantitative changes which occurred with infection suggest that the level of free fatty acid in the lung and liver is related in some way to the concentration of glycerides rather than phospholipids.

In tuberculous rabbits, by far the greatest alteration in lipid chemistry was the 60 to 70 per cent reduction in glyceride fatty acids. Available information does not seem to warrant any lengthy discussion of the possible mechanism involved. It seems worth while to mention, however, that rabbit lungs appear to participate actively in both the synthesis of fatty acids (25) and in the hydrolysis of glycerides (26). Moreover, there is suggestive evidence that tuberculous infection reduces the lipolytic activity of rabbit lung (27, 28). The reduction in glyceride fatty acids in the lungs of infected rabbits and the change in the phospholipid fatty acids in the livers of Ravenel-infected rabbits suggest that biochemical factors may play an important role in host resistance to tuberculosis in the rabbit.

Summary

A significant increase in lung weight was observed in rabbits infected with human (H37Rv) tubercle bacilli. An even greater increase occurred in rabbits infected with bovine (Ravenel) bacilli. Infection with the H37Rv strain caused a significant decrease in the percentage of fat-free residue in the liver, but not in the lung. An even greater decrease occurred in the livers of animals infected with the Ravenel strain.

The lungs of both H37Rv-infected and Ravenel-infected rabbits showed a markedly lower content of free fatty acids than did lungs of normal animals. There was no alteration in the free fatty acid content of the liver.

Experimental tuberculosis resulted in approximately a 60 per cent (H37Rv) to 70 per cent (Ravenel) reduction in glyceride fatty acids in the lungs. No qualitative alteration in the component acids was apparent. Infection did not cause any change in the glyceride fatty acid content of the liver. The saponification number of these acids was, however, decreased in rabbits infected with the H37Rv, but not the Ravenel, strain.

The concentration of phospholipid fatty acids in the lung was not altered by infection. In rabbits infected with the Ravenel strain, however, the iodine number of these acids was increased. No such change occurred in animals infected with the H37Rv strain. Calculated on the basis of milligram per gram of fresh tissue, the concentration of phospholipid fatty acids was decreased in the livers of rabbits infected with the Ravenel, but not the H37Rv, strain. No qualitative alteration in the component acids was apparent.

Sumario

Efecto de la Tuberculosis Experimental sobre la Química Lípida de los Tejidos de Conejo

En los conejos infectados con bacilos tuberculosos humanos (H37Rv), observóse un aumento significativo del peso del pulmón. El aumento fué aun mayor en los infectados con bacilos bovinos (Ravenel). La infección con la cepa H37Rv ocasionó una disminución importante en el porcentaje de residuo lipoprivo en el hígado, pero no en el pulmón. La disminución fué aun mayor en los hígados de animales infectados con la cepa de Ravenel.

Los pulmones de los conejos infectados tanto con H37Rv como con Ravenel revelaron un contenido decididamente menor de ácidos grasos libres que los de los animales normales. No hubo alteración en el contenido de ácidos grasos libres en el hígado.

La tuberculosis experimental dió por resultado una reducción aproximadamente de 60 por ciento (H37Rv) a 70 por ciento (Ravenel) en los ácidos grasos de glicéridos. No se notó alteración cuantitativa de los ácidos componentes. La infección no ocasionó cambio alguno en el contenido de ácidos grasos de glicéridos en el hígado. No obstante, disminuyó el índice de saponificación de estos ácidos en los conejos infectados con la cepa H37Rv, pero no con la Ravenel.

La concentración de ácidos grasos de fosfolípidos en el pulmón no se vió alterada por la infección. Sin embargo, en los conejos infectados con la cepa Ravenel, subió el índice de yodo de estos ácidos. No hubo tal alteración en los animales infectados con la cepa H37Rv. Calculada a base de miligramos por gramo de tejido reciente, la concentración de ácidos grasos de fosfolípidos disminuyó en los hígados de los animales infectados con la cepa Ravenel, pero no con la H37Rv. No se notó alteración cuantitativa en los ácidos componentes.

Resume

Effets de la tuberculose expérimentale sur les caractères chimiques des lipides des tissus du lapin

Une augmentation notable du poids du poumon fut observée chez des lapins infectés par des bacilles tuberculeux humains (H37Rv). Une augmentation encore plus grande s'est manifestée chez des lapins infectés par les bacilles bovins (Ravenel). L'infection par la souche H37Rv a causé une diminution significative du pourcentage du résidu en graisses libres dans le foie, mais non dans le poumon. Une diminution encore plus grande s'est produite dans le foie des animaux infectés par la souche Ravenel.

Les poumons des lapins inoculés avec le H37Rv et ceux inoculés avec la souche Ravenel présentaient une teneur nettement plus faible en acides gras libres que les poumons des animaux normaux. Il n'y avait aucune modification de la teneur du foie en acide gras libre.

La tuberculose experimentale determinait approximativement une réduction de 60% (H37Rv) à 70% (Ravenel) des acides gras des glycérides du poumon. Aucune modification qualitative de ces acides n'était apparente. L'infection ne provoquait aucun changement dans la teneur en glycéride du foie. L'indice de saponification de ces acides était abaissé chez les lapins inoculés avec le H37Rv, mais ne l'était pas pour la souche Ravenel.

La concentration des phospholipides dans le poumon n'était pas modifiée par l'infection. Toutefois, chez les lapins inoculés avec la souche Ravenel, l'indice d'iode de ces acides était augmenté. Ce changement n'apparaissait pas chez les animaux inoculés avec la souche H37Rv. Calculée sur la base d'un milligramme par gramme detissu frais, la concentration des phospholipides était abaissée dans le foie des lapins infectés avec la souche Ravenel mais ne l'était pas avec la souche H37Rv. Aucune altération qualitative des acides entrant dans la composition des lipides n'était apparente.

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THE INFLUENCE OF LIGHT UPON THE DETERMINATION OF PARA-AMINOSALICYLIC ACID CONCENTRATIONS[1]

PHILIP C. PRATT

(Received for publication June 28, 1956)

Introduction

In the course of a research project concerned with the concentrations of para-aminosalicylic acid (PAS) attained in the blood of patients treated with various forms of the drug, it was noted that there were marked variations in the concentrations attained in individual patients on different days. Moreover, there was also a marked variation in the concentrations determined within groups of patients on a single day. In some instances it appeared that there might have been a laboratory error because, repeating the analysis, it was difficult to obtain consistent results. Finally, several blood serums were analyzed repeatedly on successive days. It was found that, although the determined concentration for any given serum was seldom consistent from day to day, the relationship between various specimens was consistent and the serums would always fall in the same order if arranged from the highest to the lowest values for PAS.

After several days of this frustrating experience, it became clear that some error was affecting all serums included within any given batch of determinations. Finally, it was noted that the brightness of the day seemed to be affecting the result. Accordingly a group of specimens was analyzed simultaneously, one being in the usual area before a large window, and the other in an area of subdued light. The concentrations found when the test was done in bright light were less than half those in subdued light.

The procedure used for the determination of PAS blood concentrations at this laboratory is the colorimetric test devised by Marshall (1). It was at first presumed that the effect of light was to prevent the development or to cause fading of the azo-dye formed at the last step of the procedure following the addition of ethylene diamine dihydrochloride. However, tests were conducted to ascertain the stage of the reaction affected by light, the relationship between the original concentration of PAS and the extent of loss caused by light, and the degree of effect produced by various intensities of light. The results of these various studies form the basis of the present report.

Materials and Methods

The details of the procedure for preparing solutions and the calibration curve for determination of PAS in fluids will not be presented here because they are well described by Marshall (1). The special reagents involved are para-toluene sulfonic acid (monohydrate), sodium nitrite, ammonium sulfamate, and ethylene diamine dihydrochloride. The first reagent is a white, coarsely crystalline material. It is hygroscopic and tends to deteriorate with time and, if the stock supply is brownish in color or appears wet, it is

[1] From the Ohio Tuberculosis Hospital and the Department of Pathology, College of Medicine, The Ohio State University, Columbus 10, Ohio.

unsuitable for use. In preparing solutions, the material must be weighed rather rapidly to the nearest tenth of a gram since it is actively hygroscopic. The sodium nitrite deteriorates rapidly in solution and must be prepared fresh daily. The ethylene diamine dihydrochloride gradually develops spontaneous color and the solution should be stored in an amber bottle. It has been found convenient in this laboratory to keep all of the above solutions in the refrigerator both because it is dark and because the procedure for determination of PAS must be conducted at ice-water temperature.

For the investigations concerning the effect of light upon the results of the procedure for determination of PAS concentration, the following methods were used:

Determination of the stage of the reaction influenced by light: A floodlight[2] was set up so that the light incident upon the exposed tubes produced a reading of 1,600 on an exposure meter.[3] This entailed placement of the light at a distance of approximately two feet from the glass beaker of ice-water in which the tubes were kept. The procedure for the color reaction was then carried out in the usual four steps as follows:

(*1*) Addition of hydrochloric acid, tubes in ice bath (ten minutes);

(*2*) Addition of sodium nitrite, ice bath (five minutes);

(*3*) Addition of ammonium sulfamate, ice bath (two minutes);

(*4*) Addition of ethylene diamine dihydrochloride, room temperature (thirty minutes).

Two sets of tubes were included, each tube containing 5 ml. of a solution of PAS in a concentration of 4 γ per ml. In one set, the procedure was begun in subdued light and one tube was moved into the bright light at each step. In the second set the procedure was reversed, being started with all tubes in the bright light and with one tube moved into subdued light at each step.

Relationship of original concentration of PAS in solution to the degree of loss caused by light: To investigate the influence of the original PAS concentration, a series of standard solutions was prepared containing 0.5, 1, 2, 3, and 4 γ of PAS per ml. Two sets of these standards were prepared and analyzed, the analysis for one set being done in subdued light and for the other in natural window light at an intensity producing an exposure meter reading of 400.

The influence of various intensities of light upon the apparent concentration of PAS: For this test, the standard solution containing 4 γ of PAS per ml. was used. This solution was analyzed on several different days varying from bright sunlight to dense cloudiness. The intensity of light on the different days varied from 1,600 to 100 as measured by the exposure meter. On several occasions the determination was carried out with the Venetian blinds drawn.

Results

The effect of light upon the various stages of the color reaction used in determining PAS concentration is shown in figures 1 and 2. In each figure, the apparent concentration of PAS is plotted against the time during which the tubes were exposed to light. The steps in the procedure corresponding to the various time intervals are indicated. In figure 1 it may be seen that when there was no exposure to light (0) the procedure showed the presence of 4.18 γ of PAS per ml. Exposure to light for ten minutes after the addition of hydrochloric acid resulted in no apparent diminution of PAS concentration. However, exposure for five minutes after the addition of sodium nitrite resulted in a loss of 0.82 γ per ml.

[2] The light used was a 250 watt photographic floodlight, manufactured by the General Electric Company, in a metallic aluminum reflector. The filament was stated to function at a temperature of 3,200° Kelvin.

[3] Weston Master II Universal Exposure Meter.

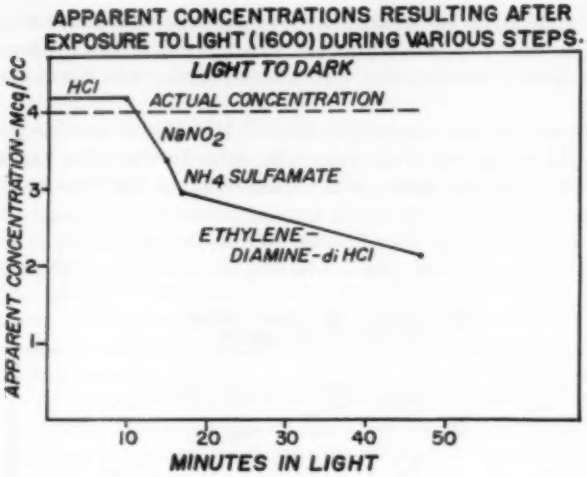

FIG. 1. Graph showing results obtained on analysis of a series of tubes each containing a solution of 4 γ of PAS per ml. The analysis was started with all tubes exposed to artificial light. As each reagent was added, as indicated, one tube was moved to an area of subdued light. The last point represents the tube which remained in bright light throughout all four steps. The horizontal spacing of the points is determined by the time allowed for completion of the reaction at each step.

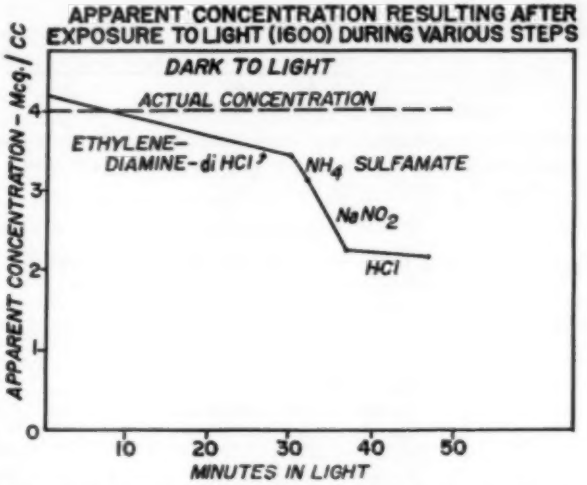

FIG. 2. Graph showing results obtained on analysis of a series of tubes each containing a solution of 4 γ of PAS per ml. The analysis was started with all tubes in subdued light. As each reagent was added, as indicated, one tube was moved into bright light. The horizontal spacing of the points is determined by the time allowed for completion of the reaction at each step.

During the ammonium sulfamate step (two minutes), another 0.41 γ was lost, and during the final step, an additional 0.85 γ was lost. When the procedure was reversed an entirely similar curve was obtained except that it was inverted (figure 2).

In figure 3 may be seen relationship between the original concentration of PAS in solution and the degree of loss caused by light. In this figure the light transmission is plotted against the original concentration of PAS. The line having the steeper slope was obtained when the determinations were conducted in subdued light. This line is the calibration curve used in this laboratory in determining PAS concentrations in blood. The line having the lesser slope represents the find-

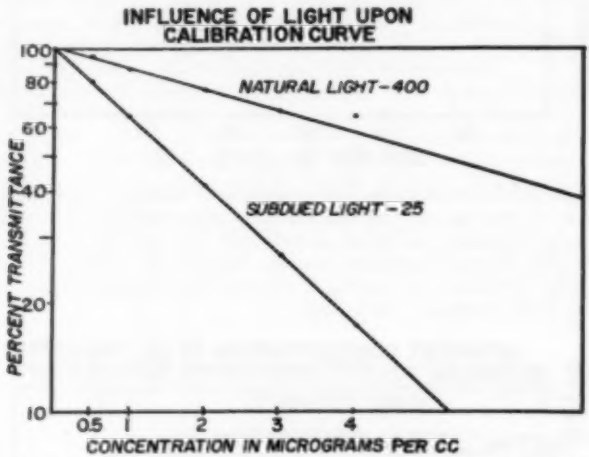

FIG. 3. Calibration curves obtained in subdued light and in moderately intense natural light, plotted semi-logarithmically. The actual percentage of transmittance obtained in subdued light with the 4 γ standard was 17.2.

TABLE 1

THE EFFECT OF NATURAL LIGHT UPON THE APPARENT CONCENTRATION OF PAS IN A STANDARD SOLUTION CONTAINING 4 γ/ML.

Intensity of Incident Light*	Description of Conditions	Apparent Concentrations
		γ per ml.
1,600	In sunlight	0.52
400	On center table, bright day	0.87
200	Cloudy bright day	2.12
100	Cloudy dull day	3.22
50	Venetian blinds drawn	3.98
35	Venetian blinds drawn	4.15
13	Venetian blinds drawn	4.15

* Reading on a Weston Master II, Universal Exposure Meter, directed towards the source of light

ings when the same standard solutions were analyzed in natural light at an intensity of 400.

In table 1 a series of results are presented which were obtained on analysis of the standard solution (4 γ per ml.) during exposure to natural light at various meter readings. Natural light at 1,600, direct sunlight, resulted in an apparent concentration of only 0.52 γ per ml. Even at a meter reading of 100 in natural light there was significant loss, but none was demonstrable at 50 or below.

Discussion

The findings presented in figures 1 and 2 show that light causes a continuous loss of apparent PAS concentration during the analytic procedure starting at the time sodium nitrite is added. The loss appears to follow an essentially straight line until the final step, which is the one in which the color development occurs. Presumably, once the color reaction has taken place, further loss of apparent concentration ceases. This fact has been confirmed by keeping the tubes for as much as forty-eight hours in natural light, at the end of which time there was no change in apparent PAS concentration.

It is clear from figure 3 that the amount of loss of apparent PAS concentration is proportional to the original concentration of PAS in solution. If this were not the case the readings obtained when the analysis was done in bright light would not have fallen on a straight line. The slight deviation of the result obtained at the 4 γ concentration suggests that the loss may be somewhat accentuated in high concentrations. However, the significant point here is that at a certain intensity of light, the apparent concentration of PAS in a solution will be halved whether the original concentration is 4 γ or 1 γ per ml.

In table 1 it may be seen that the degree of diminution of apparent concentration varies directly with the intensity of light. It is also evident from examination of table 1 and comparison with figures 1 and 2 that natural light exerts a far more pronounced effect upon apparent concentration than does artificial floodlight. Thus, while exposure to floodlight at an intensity of 1,600 throughout the analysis caused a reduction in apparent concentration from 4 γ to 2.1 γ, natural light at the same intensity caused a reduction to 0.52 γ.

Since making this observation concerning the influence of light upon this chemical reaction, it has been considered advisable to analyze a sample of the 4 γ per ml. standard each time a set of unknowns is analyzed. With the intensity of light limited by closed Venetian blinds to 50 or less, the range of results obtained in some thirty-four consecutive analyses of this standard has been between 3.80 and 4.18 γ per ml.

The error introduced by light is particularly important in the determination of PAS concentrations in blood since the specimen is first diluted 1:40 and any error in the analytic procedure is greatly magnified in the final result. As a consequence of this dilution factor, the standard solution containing 4 γ per ml. is equivalent to a blood serum concentration of 16 mg. per 100 ml. and all errors would be magnified in comparable degree.

Summary

It has been shown that light causes a loss of the apparent concentration of PAS to a degree sufficient to introduce error into the results obtained on colorimetric analysis of serum or other fluids. The effect is proportional to the amount of PAS in solution and to the intensity of light. In order to obtain consistent analyses, care should be taken to prevent exposure to excess light during the determination of concentrations of PAS in body fluids.

Sumario

El Influjo de la Luz sobre la Determinación de la Concentración de Acido Para-Aminosalicílico en los Humores Orgánicos

Queda demostrado que la luz ocasiona una pérdida de la concentración aparente del PAS en grado suficiente para viciar los resultados obtenidos con el análisis colorimétrico del suero o de otros humores. El efecto es proporcional a la cantidad de PAS en solución y a la intensidad de la luz. A fin de obtener análisis compatibles, hay que tener cuidado de impedir la exposición a la luz excesiva durante la determinación de la concentración de PAS.

Resume

Influence de la lumière sur la détermination de la concentration de l'acide para-aminosalicylique dans les liquides organiques

Il est démontré que la lumière cause une perte de la concentration apparente du PAS à un degré suffisant pour fausser les résultats obtenus dans l'analyse colorimétrique du sérum ou autre liquide organique. L'effet est proportionnel à la concentration du PAS dans la solution et à l'intensité de la lumière. Pour obtenir des analyses comparables, il y a lieu de prendre soin d'éviter l'exposition à une lumière excessive durant la détermination de la concentration du PAS.

Acknowledgment

The writer is grateful to Mrs. Greta Howard, M.T. (ASCP), for her assistance and for her key observation that light was the unknown source of error.

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THE EXPERIMENTAL PRODUCTION OF NONTUBERCULOUS CAVITIES IN RABBIT LUNGS BY EGG ALBUMIN[1]

YUICHI YAMAMURA, SHIGERU NAKAMURA, YASAKA OGAWA, AND SHIGERU YASAKA

(Received for publication April 24, 1956)

INTRODUCTION

The writers have reported previously on the method of producing tuberculous cavities in the lungs of experimental animals (1). The cavities were readily produced in normal rabbit lungs by the injection of living tubercle bacilli. When killed tubercle bacilli combined with water and paraffin oil were injected in small doses (1 mg.) no cavities formed, although positive results were obtained with large doses. However, cavities were produced when smaller doses of killed tubercle bacilli in water-in-oil emulsion were injected into previously sensitized animals (2).

These results suggested that an allergic reaction might play an important role in cavity formation, and the writers sought to isolate the active substance of tubercle bacilli which might be responsible for cavity formation. It was found that a proteolipid isolated from bacillary bodies was the primary factor involved in this reaction (3). Protein alone, isolated by Toda's method from medium in which bacilli had grown, would not in itself induce cavity formation in the normal animal, but could cause the development of cavities in the lungs of previously sensitized animals (3). These results indicated that the sensitizing substance of the bacillus responsible for the reactive state eventuating in cavity formation is a proteolipid, and that the protein portion of the bacillus can cause the reaction in the already sensitized animal. This is in accord with other works concerned with the mechanism of induction of tuberculous hypersensitivity (4–14).

The writers were encouraged by these findings to investigate the possibility that a nontuberculous allergic reaction of the same type in which egg albumin served as antigen might also result in cavity production.

METHODS

Adult male albino rabbits, weighing approximately 2.5 kg, were sensitized with egg albumin suspended in Freund's adjuvant (15). The sensitizing mixture was prepared as follows:

Egg albumin	1 gm.
Distilled water	20 ml.
Paraffin oil	40 ml.
Anhydrous lanolin	20 ml.
Heat-killed tubercle bacilli of bovine origin (Miwa strain)	100 mg. (dry weight)

One milliliter of the well-ground mixture was injected subcutaneously into the femoral region of rabbits three times at intervals of seven to ten days. By approximately the fifth

[1] From the Research Laboratory of the National Sanatorium, Toneyama Hospital; and Toneyama Institute of Tuberculosis, Osaka City University Medical School, Toyonaka, Osaka, Japan.

week after the last sensitizing injection, the rabbit serum precipitated egg albumin at dilutions greater than 1:2,000 of the antigen.

For the test injection, egg albumin was suspended in a mixture of paraffin oil and anhydrous lanolin in the following way:

Egg albumin	300 mg.
Distilled water	2 ml.
Paraffin oil	6 ml.
Anhydrous lanolin	2 ml.

One-tenth of this mixture was injected directly into the lungs of sensitized rabbits. The same amount of the mixture was injected into the lungs of nonsensitized control rabbits.

Results

Rabbits were killed and examined at various intervals after the pulmonary injections. As shown in table 1, cavities were successfully produced in the lungs of the sensitized rabbits which were sacrificed twenty-one, twenty-seven, thirty, and forty-two days after the pulmonary injection. In contrast, no cavity formation was observed in the lungs of nonsensitized animals.

The process of cavity formation by egg albumin: Twelve hours after the intrapulmonary injection of egg albumin in sensitized rabbits, marked hemorrhage and infiltration of granulocytes, mononuclear cells, and histiocytes with inflammatory fluid were observed in the injected area. This conspicuous cellular infiltration occurred through the walls of small blood vessels exhibiting stasis. The hemorrhagic inflammation reached its maximum at approximately twenty-four hours after the injection. In the surrounding area a perifocal exudative change

TABLE 1

Cavity Formation in Rabbit Lung by Egg Albumin

	Rabbit Number	Time After Pulmonary Injection	Formation of Cavity
			cm.
Sensitized rabbits	1	12 hours	—
	2	24 hours	—
	3	2 days	—
	4	5 days	—
	5	7 days	—
	6	8 days	—
	7	11 days	—
	8	15 days	—
	9	21 days	0.4 × 0.5
	10	27 days	0.6 × 0.7
	11	30 days	2.0 × 2.0
	12	42 days	0.8 × 1.0
Nonsensitized rabbits	1	2 days	—
	2	7 days	—
	3	15 days	—
	4	25 days	—
	5	30 days	—
	6	35 days	—
	7	42 days	—

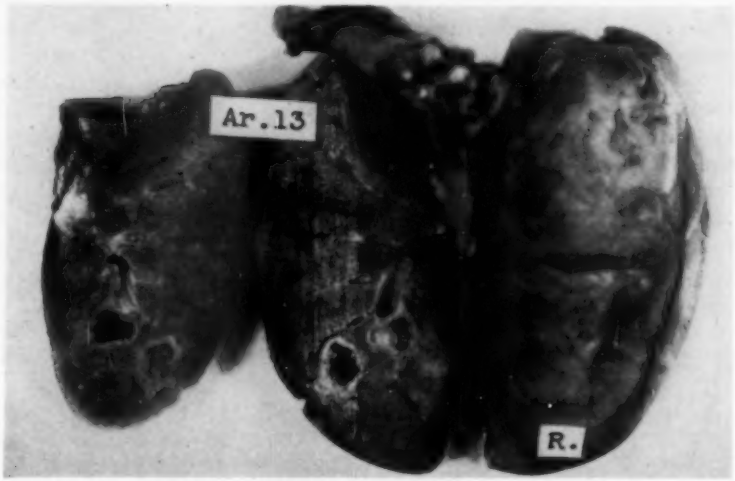

FIG. 1. Twenty-seven days after pulmonary injection of egg albumin in sensitized rabbit.

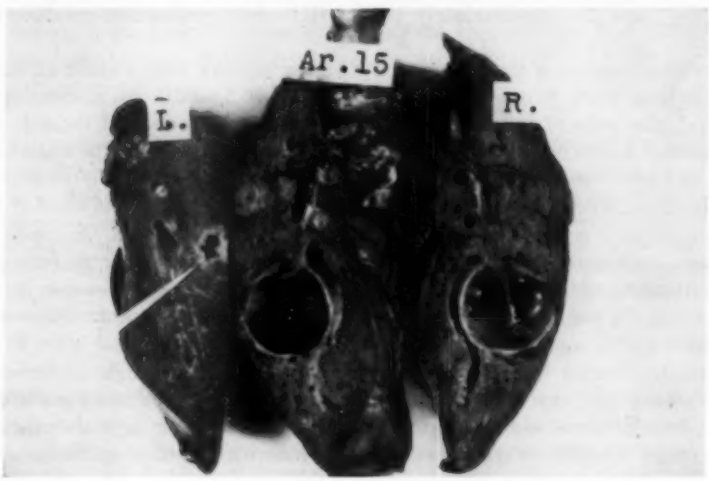

FIG. 2. Forty-two days after pulmonary injection of egg albumin in sensitized rabbit.

was taking place. As time passed, the hemorrhagic inflammation decreased and after two to five days the cellular and exudative infiltrations gave way to necrosis. From seven to eleven days after the injection, a demarcation became evident. By that time fibrous tissue had gradually developed in the periphery of the lesion and perifocal exudation had disappeared. Later, the necrotic substance became liquid and flowed out, leaving a genuine cavity at the site of necrosis

FIG. 3. Microscopic section of cavity wall. (Paraffin section; hematoxylin and eosin)

(figures 1 and 2). Approximately twenty days were required to produce cavitation.

Pathologic nature of the cavity: As a rule, the cavities were located at the site of injection. They were oval and measured from 0.5 to 2.0 cm. in diameter. In general, the walls of the cavities were thinner than the walls of those induced by tubercle bacilli or proteolipid extracted from tubercle bacilli. The walls looked pale and were covered with necrotic substance.

Histologic investigation showed that the cavity walls were composed of three layers: necrotic substance, granulation tissue, and fibrous tissue (figure 3). The necrotic substance contained many nuclear fragments, and argyrophilic fibers corresponding to alveolar septa were demonstrable by Bielschowsky's stain. Adjoining the necrosis the parenchyma was changed into a granulation tissue infiltrated with mononuclear cells, lymphocytes, and epithelioid cells. Fibrous tissues surrounded the cavity. Adjoining the lesion either slight atelectasis or emphysema was seen without any inflammatory signs. The inner walls of the cavities in rabbits killed twenty-seven, thirty, and forty-two days after the intrapulmonary injections were partially overgrown with bronchial epithelium.

DISCUSSION

In the present communication evidence is presented that when rabbits previously sensitized with egg albumin and Freund's adjuvant are given pulmonary injections with protein alone, cavities are produced after approximately twenty days. From the serial histopathologic examinations it was evident that more than two to five days were required to induce necrosis in the injected tissues. Passive transfer of antibody by injecting the sensitized serum into normal rabbits was

never successful. These results indicate that the cavities formed by egg albumin with the method described appeared on the basis of delayed hypersensitivity or tuberculin type of hypersensitivity to egg albumin.

Raffel and associates (7) reported that guinea pigs sensitized with egg albumin combined with the purified wax fraction of the tubercle bacillus respond in the skin test with delayed hypersensitivity to the protein antigen. This same mechanism presumably was operative in the present results.

The writers have previously reported that an antigen-antibody reaction in tuberculosis played an important role in the production of cavities when the proteolipid or protein extracted from tubercle bacilli was used as antigen. From the experimental result in the present study, it can be assumed that the cavity formation is demonstrated, not only by tuberculous antigen but also by nontuberculous antigen such as egg albumin, on the basis of delayed hypersensitivity.

Summary

Egg albumin, emulsified in paraffin oil and anhydrous lanolin, was injected into the lungs of rabbits sensitized to this protein by previous injections employing Freund's adjuvant containing killed tubercle bacilli. Cavities resulted in the lungs of sensitized rabbits which were killed twenty-one, twenty-seven, thirty, and forty-two days after intrapulmonary injection. No cavity formation was observed in the lungs of nonsensitized rabbits. It is assumed that the cavity is produced on the basis of a delayed hypersensitivity.

Sumario

La Producción Experimental de Cavernas en los Pulmones de Conejos por Ovalbúmina

Inyectóse ovalbúmina, emulsificada en aceite de parafina y lanolina anhidra, en los pulmones de conejos sensibilizados a dicha proteína por inyecciones anteteriores en que se empleó el coadyuvante de Freund que contenía bacilos tuberculosos muertos. Esto dió por resultado cavernas en los pulmones de conejos sensibilizados y matados a los veintiún, veintisiete, treinta y cuarenta y dos días de la inyección intrapulmonar. No se observó formación de cavernas en los pulmones de conejos no sensibilizados. Se presupone que la caverna se forma a base de una hipersensibilidad retardada.

Resume

Production expérimentale d'excavations dans les poumons du lapin par l'albumine d'oeuf

De l'albumine d'oeuf, émulsifiée dans l'huile de paraffine et la lanoline anhydre, fut injectée dans les poumons de lapins sensibilisés à cette protéine par des injections antérieures d'adjuvant de Freund renfermant des bacilles tuberculeux tués. Il s'est produit des cavernes dans les poumons des lapins sensibilisés qui étaient tués vingt-et-un, vingt-sept, trente et quarante-deux jours après l'injection intrapulmonaire. Aucune formation cavitaire ne fut observée dans les poumons des lapins non sensibilisés. Les auteurs estiment que la production de l'excavation est determinée par une hypersensibilité tardive.

Acknowledgment

The writers wish to express their gratitude to Professor Sidney Raffel, Department of Medical Microbiology, Stanford University, California, for his advice and interest in this work.

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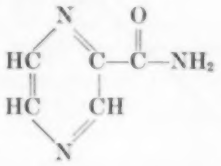
SPECTROPHOTOMETRIC DETERMINATION OF PYRAZINAMIDE BLOOD CONCENTRATIONS AND EXCRETION THROUGH THE KIDNEYS[1]

P. A. CACCIA

(Received for publication May 17, 1956)

Introduction

Pyrazinamide is the amide of pyrazinoic acid from pyrazine (1-4 diazine) having the following structural formula:



Pyrazinamide has been found useful in pulmonary tuberculosis but, because of its toxicity and of its rapid disappearance from the blood stream, its quantitative determination in body fluids from patients under treatment becomes a necessity (1–3). A calorimetric method has been described for the determination of the amount of pyrazinamide in blood and urine (4).

The method is based on the fact that pyrazinamide reacts with sodium nitritopentacyanoferroate in strongly alkaline medium to give a red-orange-colored complex, whose color intensity can be determined spectrophotometrically at 500 wavelength (figure 1). The color develops instantly and is stable for hours. It follows Beer's Law, from 5 to 100 γ per milliliter with a deviation from 100 to 200 γ. The method is original for pyrazinamide (4). With the use of this method, quantitative studies of the distribution of pyrazinamide have been made, and the results form the basis for the present report.

Materials and Methods

Preparation of pyrazinamide standard: Forty milligrams of pyrazinamide were dissolved in water and made to a volume of 100 ml. in a 100 ml. volumetric flask. For a working standard, 10 ml. of the stock standard were pipetted into a 100 ml. volumetric flask and made to mark with distilled water. One milliliter contains 40 γ of pyrazinamide.

Determination of pyrazinamide in water solution: Pyrazinamide follows Beer's Law, deviating slightly as the concentration reaches 200 γ per milliliter. Determination of pyrazinamide when present in concentrations ranging from 10 to 200 γ per ml. is as follows:

> Twenty-five milliliters of the stock standard were pipetted into a 100 ml. volumetric flask and made to mark with distilled water; 1 ml. of the above solution contained 100 γ of pyrazinamide. A set of cuvettes received 10, 25, 50, 75, 100, 150, and 200 γ of pyrazinamide from the standard solution. By the addition of water the amount of fluid in each cuvette was brought to 2 ml. Each cuvette

[1] From the Veterans Administration Hospital, Sunmount, New York.

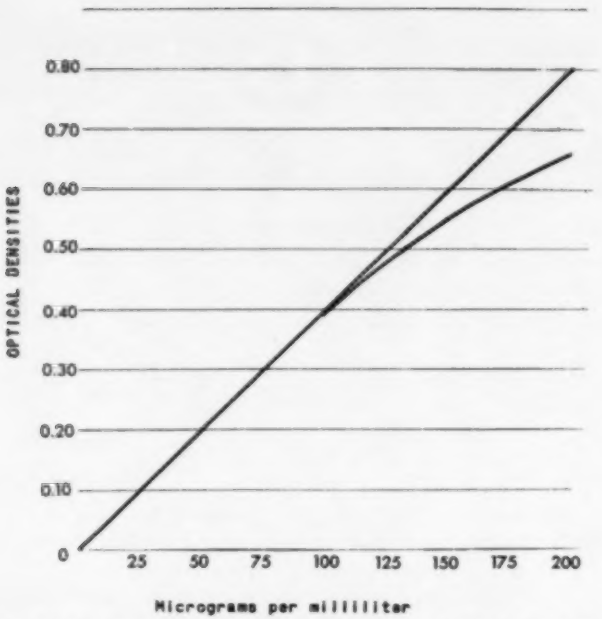


FIG. 1. Beer's Law deviation of pyrazinamide in water solution. Readings at spectrophotometer at 500 wavelength.

received 1 ml. of reagent freshly prepared and 7 ml. of water to bring the total volume to 10 ml. The blank received 9 ml. of water and 1 ml. of reagent. Each cuvette was mixed and allowed to stand at room temperature for ten minutes, and was then read at the spectrophotometer at 500 wavelength; the blank at 0 optical density.

10 γ read: 0.04	75 γ read: 0.310
25 γ read: 0.11	100 γ read: 0.395
50 γ read: 0.21	150 γ read: 0.560
	200 γ read: 0.660

The above were averages of many readings.

Method for determination of pyrazinamide in blood serum: In determining the pyrazinamide content of blood, the blood was centrifuged and 2 ml. of serum were pipetted into a test tube. Two milliliters of $\frac{2}{3}$ N sulfuric acid and 2 ml. of 10 per cent sodium tungstate were added, mixed well, and centrifuged for fifteen minutes at 3,000 r.p.m. A clear supernatant was obtained. (When particles appeared on the top, the solution was shaken and centrifuged again for five minutes.) The clear supernatant was poured into test tubes. Three milliliters of the clear fluid, equivalent to 1 ml. of the original blood serum, were pipetted into a cuvette. The working standard, 1 ml. containg 40 γ per ml., or 0.5 ml. containing 20 γ, was pipetted into a cuvette and enough water was added to make 1 ml. of the color reagent freshly prepared and brought to the 10 ml. mark by adding water. Each cuvette was mixed and allowed to stand at room temperature for ten minutes. The color complex developed instantly from light orange to red-orange according to the amount of pyrazinamide, as

Beer's Law requires. The reading was done at the spectrophotometer, the blank at 0 optical density and 500 wavelength. The blank was yellow in color.

Observations

Pyrazinamide concentrations in the blood: The maximal amount of pyrazinamide in the blood has been found at or about two hours after the ingestion of the drug. A patient received 1 gm. of pyrazinamide orally, and after two hours the amount of pyrazinamide in the serum was 45 γ per ml. The same patient received 1 gm. of pyrazinamide at night and in the morning, after fifteen hours, showed a serum blood concentration of 10 γ per ml. Two patients received the nightly dose of 0.5 gm. of pyrazinamide and in the morning, after fifteen hours, showed a blood concentration of 5 γ per ml.

Determination of pyrazinamide added to blood serum: A standard solution of pyrazinamide was added to serum, was well mixed, and then was treated as described for blood serum. The results were as follows:

Added γ per ml. Blank: Serum	Found γ per ml. 0
10 γ	9.7 γ
25 γ	24.8 γ
50 γ	49.6 γ
100 γ	99.0 γ

Determination of pyrazinamide in urine: Pyrazinamide in solution is adsorbed totally by activated charcoal. With a 10 ml. mark, 1 ml. of urine diluted 1:10 with water was pipetted into cuvettes, and 1 ml. of the dilution was pipetted into eight small beakers. To the cuvettes and to the beakers, the following amounts of a standard solution of pyrazinamide containing 200 γ per ml. were added: the set of cuvettes received 10, 25, 50, 75, 100, 150, and 200 γ of pyrazinamide from the standard solution, the eighth cuvette being the blank. The set of beakers received the same amounts. To each beaker, about 100 mg. of activated wood charcoal were added and to the sixth and seventh beakers, about 200 mg. The mixture was brought to a liquid paste with the addition of a little water when necessary and then 3 ml. of water were added to each beaker, filtered through small filter paper into cuvettes marked to 10 ml. The contents of the beakers were washed with water, using a wash bottle with a fine jet. Approximately 8 ml. of filtrate were collected. Water was added to the first set of cuvettes to bring about the same volume as that of the second set. To each of the 16 cuvettes, 1 ml. of color reagent freshly made was added, mixed, let stand for ten minutes, and was then read at the spectrophotometer at 500 wavelength. The blank was at 0 optical density.

Reading of the first set	γ added	γ found	Reading of the second set	γ found
1—0.05	10	12	1—0	0
2—0.11	25	25	2—0	0
3—0.22	50	55	3—0	0
4—0.32	75	77	4—0	0
5—0.42	100	106	5—0	0
6—0.56	150	152	6—002	0.5
7—0.72	200	212	7—005	1.1

As may be seen from the above experiment, pyrazinamide was totally adsorbed by charcoal. The recoveries from the first set compared favorably with the curve; the amounts of pyrazinamide found were slightly higher, but that was to be expected because the curve had been obtained by averages.

Before any determination of pyrazinamide is performed, it is advisable to test the urine for other chemicals giving the same reaction as pyrazinamide. If these chemicals are not adsorbed by charcoal, then the color reaction takes place in the filtrate. By using 1 mg. or more of charcoal per γ of pyrazinamide, this chemical is completely adsorbed. Urea is an example of a chemical not adsorbed by charcoal. Urea gives a color complex with the reagent only on standing for more than one hour and when present in a very large amount.

The following example shows that almost 100 per cent of the urea can be recovered and determined in the filtrate:

Into a small beaker, containing 2 ml. of water, 100 mg. of urea and 200 γ of pyrazinamide were added. Two hundred milligrams of activated wood charcoal were added, mixed to a liquid paste, and then pipetted in approximately 3 ml. of water. The mixture was filtered through a small filter paper (the beaker was washed well with a fine jet of water from a wash bottle) and was added to the filter. Ten milliliters of filtrate were collected. The filtrate was mixed by shaking, and the determination of urea was made in the usual way by the urease method. One milliliter of filtrate gave a recovery of 9.6 (96 per cent) of the original urea added.

Cycloserine also is not adsorbed by charcoal and can be determined in the filtrate. When a patient is treated with the two drugs, the pyrazinamide color reaction interferes with the reaction of cycloserine. In this case, pyrazinamide is adsorbed by charcoal, and the determination of cycloserine can be performed in the filtrate.

Determination of pyrazinamide added to urine: The urine was diluted 1:10. To each cuvette, 1 ml. of color reagent was added, and water was added to the 10 ml. mark.

Added γ per ml.	Found γ per ml.	Spectrophotometric Reading Optical Density
10	11	0.045
25	27	0.120
50	50	0.210
75	77	0.320
100	100	0.395
150	152	0.560
200	200	0.680

Blank of urine equally treated, 0 optical density.

The above were averages of many readings obtained from urines of several patients and of normal subjects.

Excretion of pyrazinamide through the kidneys: Judging by the results of all the determinations made in this laboratory, pyrazinamide is eliminated through the kidneys in large amounts, as the following example will demonstrate:

A patient received orally 1 gm. of pyrazinamide at 7:30 a.m. Four samples of urine were collected at one-hour intervals, also a sample of urine from a patient who had never received pyrazinamide. The urine was diluted 1:10 with water. Five cuvettes each received 1 ml. of the diluted urine samples; and one cuvette (the blank) received 1 ml. of water. Each cuvette also received 1 ml. of the pyrazinamide color reagent and 8 ml. of water to a total volume of

10 ml. After mixing, the reading was made at the spectrophotometer at 500 wavelength after standing ten minutes at room temperature, the blank at 0 optical density:

1-hour sample − 0.33 =	82 γ × 10 × 80	(ml. of urine) =	65.6 mg.
2-hour sample − 0.27 =	67 γ × 10 × 97	(ml. of urine) =	65.0 mg.
3-hour sample − 0.49 =	126 γ × 10 × 29	(ml. of urine) =	36.5 mg.
4-hour sample − 0.18 =	45 γ × 10 × 115	(ml. of urine) =	52.0 mg.
		Total	219.0 mg.

5 cuvette blank—sample of normal urine, 0 optical density.

In four hours the kidneys had excreted 219 mg. of pyrazinamide. The above data were calculated from the curve.

Sensitivity of the test: Three sets of cuvettes received the following amounts of pyrazinamide:

	Reading at 500 wavelength	Found
1—10 γ	0.040	10.0
2—12 γ	0.048	12.0
3—14 γ	0.060	15.0
4—25 γ	0.110	25.0
5—30 γ	0.130	29.5
6—35 γ	0.156	35.4
7—50 γ	0.206	50.0
8—52 γ	0.216	52.4
9—54 γ	0.237	57.7

The sensitivity of the test was about 0.5 to 2.0 γ per ml.

Interference with the assay of pyrazinamide: The chemicals in the following list were tested and none gave the pyrazinamide color reaction: streptomycin, para-aminosalicylic acid (PAS), cycloserine, adenine, guanine, sodium nucleate, aspartic acid, glutamic acid, cystine, arginine, glycine, histidine, lysine, methionine, phenylalanine, hydroxyproline, proline, and uric acid.

There was a slight colored complex formed by urea after it had been standing from one to several hours. No colored complex was noted after ten minutes when 50 and 100 mg. of urea were added to urine or to water in the 10 ml. cuvette. On standing, a slight orange color started to appear after one hour, and increased after one hour. Thus, no complications from this source are to be expected when tests are done at ten-minute intervals. Isoniazid gave a slight color complex, also orange like pyrazinamide. Its color intensity was so weak that 100 γ per ml. gave a spectrophotometric reading of 0.013 equivalent to 3 γ of pyrazinamide per ml.

Summary

A colorimetric method for the determination of pyrazinamide in blood and urine has been described. The color is stable for hours. The sensitivity of the test approaches 0.5 to 2.0 γ per ml. Pyrazinamide is adsorbed by activated charcoal. Blood concentrations reach a maximum in approximately two hours. Large amounts of pyrazinamide are excreted through the kidneys.

Sumario

Determinación Espectrofotométrica de la Piracinamida (PCA). Concentraciones Sanguíneas y Excreción Transrrenal

Se describe aquí un método colorimétrico para la determinación de la piracinamida en la sangre y la orina. El color es estable horas enteras. La sensibilidad de la prueba se aproxima a 0.5 a 2.0 por ml. La piracinamida es adsorbida por el carbón vegetal activado. Las concentraciones sanguíneas alcanzan su máximo en unas dos horas. Por los riñones se excretan grandes cantidades de la droga.

Resume

Détermination spectrophotométrique de la pyrazinamide. Concentrations sanguines et excrétion par le rein

Description d'une méthode colorimétrique pour la détermination de la pyrazinamide dans le sang et l'urine. La coloration est stable pendant des heures. La sensibilité du test est de l'ordre de 0, 5 à 2 par ml. La pyrazinamide est adsorbée par le charbon acti"é. Les concentration sanguines atteignent un maximum en approximativement deux heures. Une quantité importante de la pyrazinamide est excrétée par les reins.

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Case Reports

CHRONIC PULMONARY HISTOPLASMOSIS COMPLICATED BY PREGNANCY AND SPONTANEOUS PNEUMOTHORAX[1, 2, 3]

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Introduction

Histoplasmosis has been a known medical entity for only fifty years. During most of this period it was diagnosed chiefly by post-mortem examination. The impression grew that histoplasmosis was almost invariably fatal, and knowledge of the morbid condition was limited for the most part by the small number of cases that came to autopsy. When it became apparent some ten years ago following the studies of Christie and Peterson (1) that infection with the causative agent, *Histoplasma capsulatum*, was quite prevalent in certain areas of the United States, it was assumed that the disease histoplasmosis was a good deal more common than had been supposed. It was not unreasonable to assume also that various gradations of the disease might be encountered, ranging from benign asymptomatic infection to fatal disseminated miliary histoplasmosis. That these assumptions were valid is evidenced by the fact that many more cases of the disease have been diagnosed during the past decade than during the first forty years of its known existence. Moreover, clinicians are now encountering and recognizing a great variety of manifestations of histoplasmosis. The disease is no longer a medical curiosity in some sections of the country, but knowledge of its variations and complications is still far from complete.

The case here reported manifests two complications that have not been reported previously so far as is known. The patient of the present report has experienced four pregnancies and has had two episodes of spontaneous pneumothorax during the eight years she is known to have had pulmonary histoplasmosis.

Case Report

R. C., a white married female, thirty-one years of age, has lived all her life in a rural agricultural environment in middle Tennessee. In 1940, at the age of fifteen, she had a chest film taken as part of the required examination for food handlers. This film showed several small calcified foci in the upper portion of the left lung, with calcification of tracheobronchial glands in the left hilar region. It was otherwise not remarkable. In 1945, a 70 mm. survey film showed no change.

The patient first became symptomatic in the autumn of 1948 when she developed respiratory complaints which she attributed to a "cold." She had symptoms of fatigue, cough, and expectoration of odorless mucopurulent sputum. In October, 1948, physical signs were meager, but a chest film showed nodular infiltration in the upper third of the left lung. She was suspected of having pulmonary tuberculosis and was requested to return for diagnostic

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[1] From the Tennessee Department of Public Health; and the Department of Preventive Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee.

[2] Presented, in part, before the Medical Session, as part of *Section 2A*, at the annual meeting of the National Tuberculosis Association, New York, New York, May 21, 1956.

[3] This investigation was supported in part by a grant (E-521) from the National Microbiological Institute of the National Institutes of Health, Public Health Service.

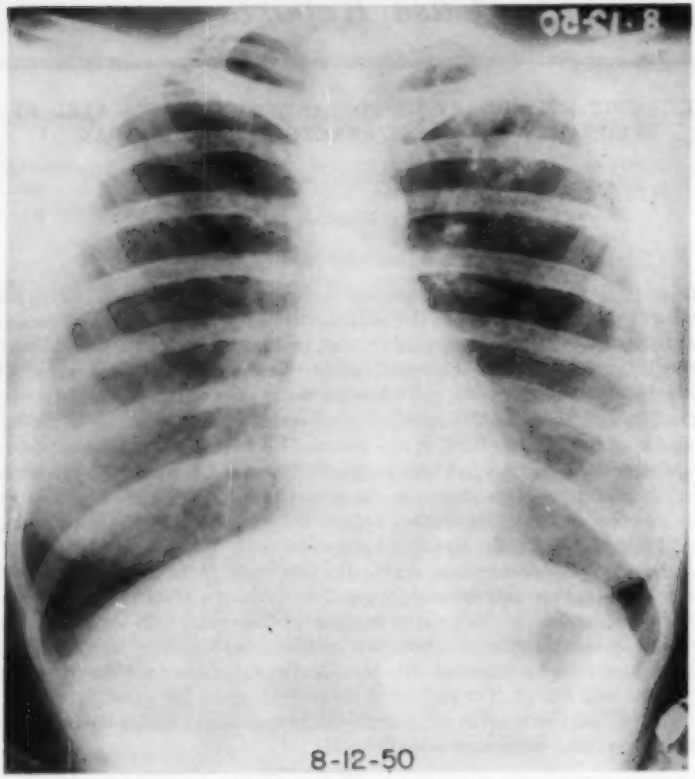

FIG. 1. Roentgenogram showing fine infiltration throughout most of the right lung; infiltration in the left upper lung field with a cavity in the first interspace.

studies, but she failed to do so until August 12, 1950, almost two years later. In the interval she had experienced her first pregnancy, which was full term and was reported to have been uneventful.

The patient's first child was born on April 21, 1950. Following delivery, the cough, expectoration, and fatigue became more pronounced and, in addition, she began to lose weight and developed hoarseness and dyspnea. When seen on August 12, 1950, she appeared pale, undernourished, and chronically ill. Her weight was 12 pounds less than that recorded in 1948. Other significant physical findings were limited to the chest and revealed dullness, bronchovesicular breathing, and coarse moist rales over the upper third of the left lung. A chest film (figure 1) showed fine infiltration throughout most of the right lung. On the left there was nodular infiltration from apex to the third anterior rib, with a 1 by 2 cm. cavity in the first interspace. An intradermal test with Old Tuberculin (OT), 0.01 mg., on September 2, 1950, was read in forty-eight hours as negative. A histoplasmin test (Christie antigen, 1:100, 0.1 ml.) made at the same time showed a 1-plus reaction. Three sputum smears were negative for acid-fast bacilli. A second, stronger intradermal tuberculin test (OT, 1.0 mg.) was made soon after, and this too was negative in forty-eight hours. Three sputum specimens were examined for acid-fast bacilli in September, 1950, and three more

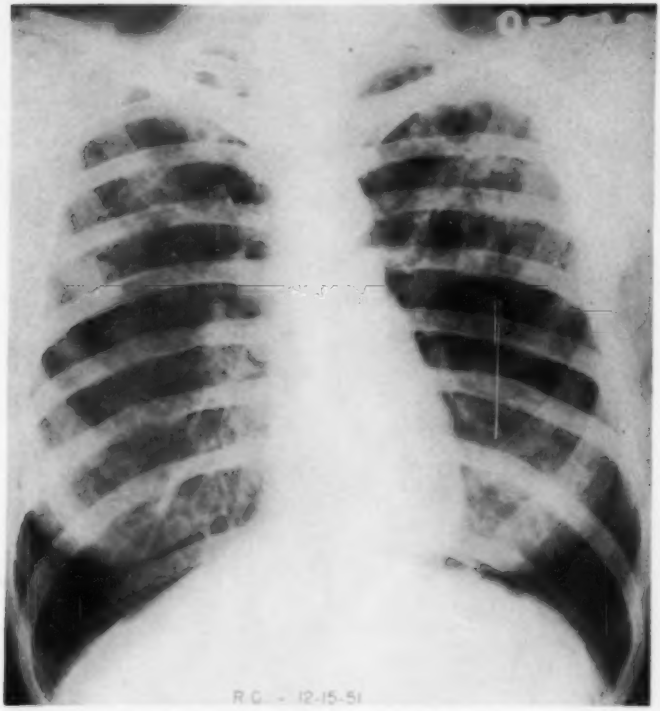

FIG. 2. Roentgenogram showing infiltration extending to the fourth anterior rib bilaterally; cavities in the first interspace on the right and in the first and second interspaces on the left.

in October, 1950. All were reported negative. In view of the negative tuberculin reactions, it was considered reasonably certain that this patient did not have tuberculosis, but histoplasmosis was suspected.

Her course was progressively downhill. A small infiltrate developed in the right upper lung, while the lesions on the left extended and the cavity became larger. By December, 1951, her condition was very poor. Her weight loss totaled 19 pounds, and further extension of the disease was noted in both lungs (figure 2). A cavity was now apparent in the right apex and two cavities were present on the left. Sputum smears were still negative for acid-fast bacilli, as was a sputum culture, but *Candida albicans* was isolated from one sputum specimen. This finding was not considered pathognomonic. The patient received symptomatic therapy from a local physician without improvement in her condition.

A histoplasmosis complement-fixation test was positive in a dilution of 1:16 (whole yeast phase antigen) on January 26, 1952. This, together with the positive histoplasmin skin test, the pulmonary pathology, and the patient's residence in an area of high prevalence of histoplasmosis, added up to presumptive evidence in support of such a diagnosis, but could not be considered as proof. Conclusive evidence was soon available however. In February, 1952, the complement-fixation titer rose to 1:40, and the first of several isolations of *H. capsulatum* was obtained by culture of the sputum. A soil sample taken from inside a chicken house on

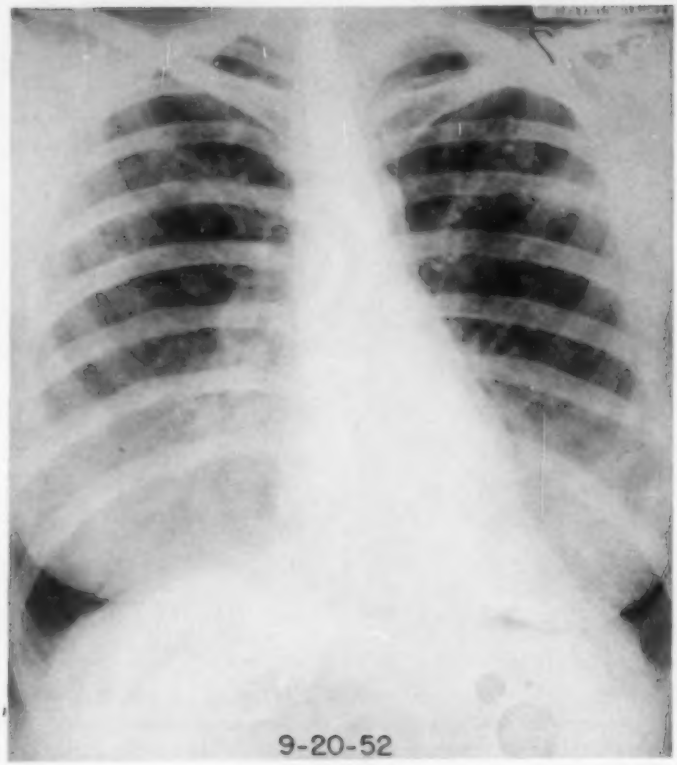

FIG. 3. Roentgenogram showing shrinkage of lesions in both lungs; cavities smaller. Obtained during second pregnancy, seventh month.

the patient's homesite yielded *H. capsulatum* on mouse inoculation. The complement-fixation titer eventually rose to 1:80, where it has remained fairly well fixed for several years. Three more sputum cultures were positive for *H. capsulatum* in 1952.

Following the second pregnancy the patient's course worsened again. She lost 35 pounds in nine months and her pulmonary lesions became more extensive, particularly on the left where the cavity in the second interspace showed a notable increase in size and infiltration extended down to the fifth anterior rib.

In January, 1954, she developed bronchopneumonia in the right base but this cleared promptly with antimicrobial therapy. She was admitted to Vanderbilt University Hospital for study on April 22, 1954, and remained for about two weeks. The etiology of the pulmonary disease having been definitely established, it was desired to know whether the histoplasmosis had remained localized in the lungs or whether dissemination had occurred. Accordingly, tests of hepatic, renal, and adrenal function were done, but these showed no evidence of impairment. Vital capacity was 98 per cent of normal and respiratory function was surprisingly good, considering that the chest appeared to be somewhat emphysematous, and the pathologic involvement of both lungs was moderately extensive. Cultures of sternal marrow, blood, and sputum failed to reveal either acid-fast bacilli or fungi.

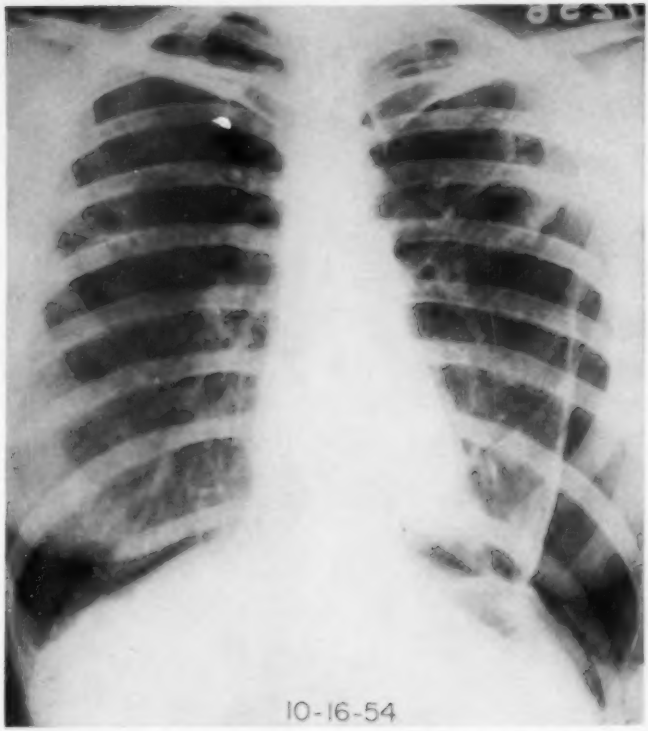

FIG. 4. Roentgenogram showing spontaneous pneumothorax on the left; no fluid. Lesions in both lungs are smaller; no evidence of cavity in the first interspace on the left. Obtained during third pregnancy, third month.

The patient became pregnant for the third time in July, 1954, and once again her general condition improved. In October, 1954, while stretching to reach a dishpan hanging over the kitchen sink, she developed a sudden sharp pain on the left side of the chest and severe dyspnea. A chest film on October 16, 1954 (figure 4) showed about 25 per cent collapse of the left lung which was adherent to the parietal pleura in the apex and base. A cavity was visible in the second interspace and some fibrosis was present below it. On the right a cavity was noted in the third interspace. No fluid developed and the lung re-expanded without complication. On January 3, 1955, when the patient was in the sixth month of pregnancy, a film (figure 5) showed only some residual fibrosis on the right and a small fibrocavernous lesion in the second interspace on the left. The third child was full term, born without incident on April 6, 1955, after a normal labor. Following this pregnancy the usual course was observed. The patient lost more than 30 pounds in a few months and her symptoms became more severe. She began to experience difficulty with her breathing which became wheezy. Although no sensitivity studies were done, it did not appear likely that her difficulties were due to allergy. Her wheezing and brassy cough became more severe in association with recurrent respiratory infections.

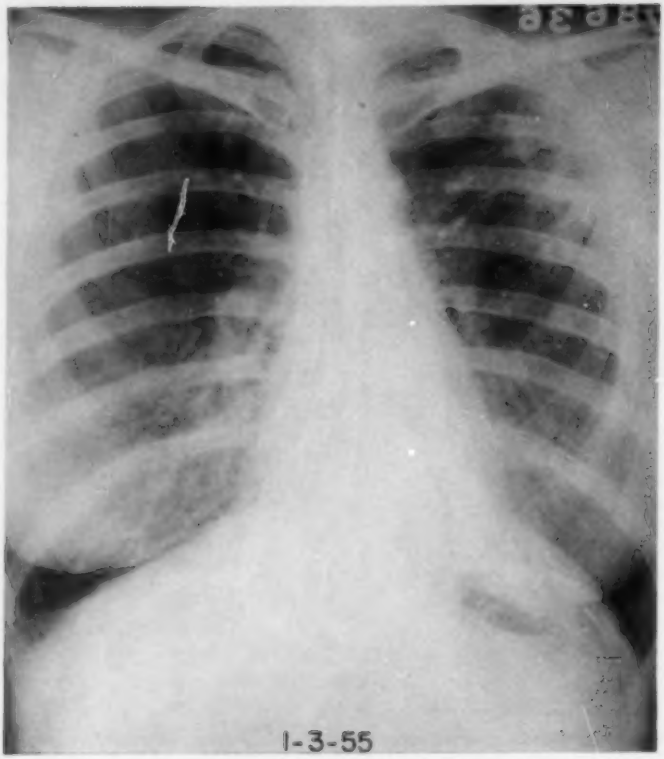

FIG. 5. Roentgenogram showing complete re-expansion of left lung; no pleural reaction. Small amount of fibrosis present in right lung with no evidence of cavity; lesions on left smaller. Obtained during third pregnancy, sixth month.

The patient became pregnant for the fourth time in June, 1955. She did poorly during the early months of this pregnancy. On August 11, 1955, she presented herself at the clinic with a complaint of severe dyspnea and chest pain of recent onset. A chest film taken at this time (figure 6) showed a small pneumothorax on the right with only about 10 per cent collapse. However, the heart and mediastinum were shifted slightly to the left. Only a small amount of fibrosis was present in the right upper lung field, but on the left there was a large cavity in the second interspace with dense infiltration involving most of the upper half of the lung. The right lung subsequently re-expanded without incident and the patient improved clinically. She was delivered of her fourth child on March 24, 1956, after a normal labor.

The placenta, maternal blood, and cord blood were obtained at the third and fourth deliveries. In both instances the placenta appeared normal grossly and microscopically. Cultures for *H. capsulatum* were negative. The cord blood taken from the third and fourth infants born to this patient revealed the presence of histoplasmosis complement-fixing antibodies. A more detailed report of these studies will be made later.

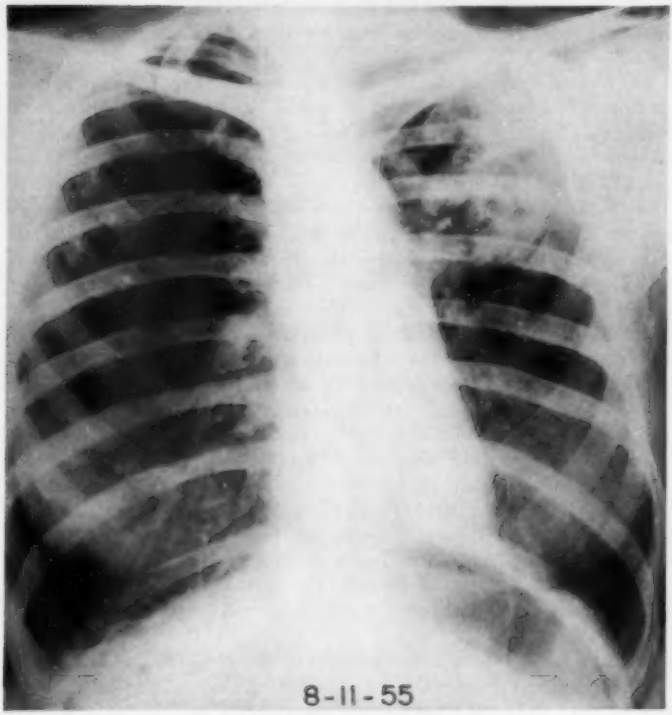

FIG. 6. Roentgenogram showing small spontaneous pneumothorax on the right; slight mediastinal shift to the left. Right lung is fairly clear; left lung shows dense infiltration surrounding a cavity in the second interspace. Obtained during fourth pregnancy, second month.

COMMENT

This patient with histoplasmosis, who has been under observation for eight years, presents several interesting features. In the first place, the long duration of her illness merits some comment. It is only within the past few years that the chronic cavitary form of pulmonary histoplasmosis has been recognized, and insufficient time has elapsed for the accumulation of knowledge concerning the long-term course and prognosis of the disease. In 1951, Hodgson (2) reviewed 138 cases of pulmonary histoplasmosis, 65 of them proved, among which the longest recorded survival time was five years. Monroe and Kurung (3) reported a confirmed case of histoplasmosis in 1953 that they believed had had its onset in 1939, but the protocol is not conclusive, and the writers themselves state that "... the onset of the present illness might well be dated as of March, 1951." Sutliff and associates (4) list the case of a farmer who had symptoms of pulmonary disease twenty years before his current observation for histoplasmosis, and they speculate on the possibility that the initial complaints marked the beginning of his histoplasmosis. However, the original diagnosis was tuberculosis, and acid-fast bacilli were present in his sputum at

that time. More recently Furcolow and Brasher (5) reported a series of cases of cavitary histoplasmosis, two of which came under observation in 1948, and the others more recently.

These few reports represent the recorded instances of "long-term" pulmonary histoplasmosis from which our knowledge of its course can be drawn. Obviously, sweeping generalizations are hardly valid. It is much too early to label this type of chronic cavitary histoplasmosis "progressive," as Furcolow and Brasher have done. In their own series, more than half of the patients showed no change or were improved on last roentgenographic observation. The patient presented here manifested "progressive" disease at times to be sure, but it is has been as often "regressive." At the present time, with the meager information on hand based on few observations, there is no evidence that cavitary histoplasmosis follows a characteristic progressive course, and to label it that way is misleading. In the absence of specific therapy there is an opportunity to study the natural course of the disease in these patients. It would not be surprising to find that in some of them the disease improves and even becomes arrested, if not healed, while in others it may progress to a fatal issue. If the parallel of histoplasmosis and tuberculosis holds, many variations in course and uncertainty of prognosis may be expected.

The subject of this report manifested parenchymal and tracheobronchial lymph node calcification in 1940, when her lungs were otherwise clear. In the light of subsequent negative tuberculin reactions and her residence in an area of high prevalence of histoplasma infections, it may be assumed that her initial primary complex was caused by the fungus. If this is so, her subsequent pulmonary disease may be looked upon as a reinfection type of histoplasmosis, perhaps similar in pathogenesis to reinfection tuberculosis, and further evidence of the similarity of the two diseases. The development of lesions on the right side probably was caused by a bronchogenic spread of infected sputum which originated in the cavities on the left.

Of particular interest is this patient's course of disease in relation to her pregnancies. Periods of improvement appeared to coincide with periods of gestation. Exacerbations commonly followed delivery. Whether this was purely a coincidence or whether it was significant may have a bearing on possible therapy. Three likely explanations of her improvement during pregnancy come to mind. The first of these is that abdominal pressure exerted on the diaphragm by the enlarged uterus had a beneficial effect similar to that observed by the use of pneumoperitoneum in some types of tuberculosis. However, no great elevation of the diaphragm was evident on this patient's films during pregnancy. Nevertheless, it might be worth while to try pneumoperitoneum for the treatment of cavitary histoplasmosis, particularly since specific therapy for the disease is not currently available.

A second possibility is that the improvement during gestation might have been caused by hormonal factors. In experimental studies on mice, Mankowski (6) has shown that animals infected with *H. capsulatum* died after the administration of estrogenic hormones while controls survived. This was particularly true for female mice. Progesterone alone had no effect on the disease, but when administered with estrogens it showed a remarkable capacity to counteract or inhibit the effect of the latter. Corticotropin is also known to have a deleterious effect on many fungal diseases, including histoplasmosis. Since all three of these hormones tend to increase in the circulation during pregnancy, the effect on the disease should have been far different from the course observed in this patient.

A third possibility is that the anabolic processes of pregnancy had a beneficial effect and might have been responsible for the remissions of the disease. Similar reactions have

been observed in other morbid conditions, including tuberculosis. At best this is only a superficial explanation.

The two episodes of spontaneous pneumothorax experienced by this patient are an unique complication of pulmonary histoplasmosis. A search of the literature fails to reveal any account of a similar episode, although Puckett (7) reported two cases in which single small isolated lesions of histoplasmosis were found in the lungs removed for treatment of other conditions in patients who had given a history of spontaneous pneumothorax in the past. Puckett did not believe, however, that the histoplasmosis was responsible for the pneumothorax. In the present case also, it does not seem likely that the pneumothorax was caused directly by the fungal disease in the lungs. If a superficial lesion had ruptured, empyema probably would have been inevitable. Furthermore, delayed re-expansion of the lung, if not the development of a bronchopleural fistula, might have been expected. Instead, no sequelae were observed, and re-expansion of the lungs occurred quite rapidly. It must be inferred, therefore, that the collapse of the lungs was caused in each instance by the rupture of a superficial bleb.

The patient of the present report had some degree of fibrosis and distortion of pulmonary tissue resulting from the healing of some of her lesions. In addition, she suffered also from an asthmatic type of chronic bronchitis which is frequently associated with emphysema of varying degree. Future episodes of spontaneous pneumothorax may be anticipated. In any case, her experience did her no harm. Coming during pregnancy as they did, the two episodes of pneumothorax occurred at a time when the patient's condition was improving. It would be difficult to assess what part, if any, these episodes played in the remissions, but they could not have influenced the course appreciably because they were of such small degree and of such short duration.

The question of placental involvement and placental transmission of histoplasmosis came under consideration in the light of a report by Randall and associates (8) of the presence of organisms resembling yeast forms of *H. capsulatum* in tissue culture of amnio-allantoic membrane obtained from a gravid mare. In the patient presented here, placentas obtained at the last two deliveries were normal anatomically and sterile mycologically. Since histoplasmosis may be disseminated hematogenously, it is entirely within the realm of possibility that infection of the human placenta may sometimes occur. There has been no evidence of congenital infection of the infants at birth. Histoplasmin tests made on the last two children soon after their birth were negative, although both had histoplasmosis complement-fixing antibodies in their circulating blood.

Summary

A case report is presented of a 31-year-old white female who has had known pulmonary histoplasmosis for eight years and who has had four full-term pregnancies during this period. With gestation the disease tended to improve and, following delivery, lesions usually spread, cavities increased in number and size, and the patient lost considerable weight. She experienced two episodes of spontaneous pneumothorax, once on each side, but recovered without complications or sequelae.

The long duration of the disease in this patient is discussed against the background of the paucity of information concerning the long-term course and prognosis of chronic cavitary histoplasmosis.

Three possible explanations of the patient's improvement during pregnancy are offered, and it is suggested that pneumoperitoneum therapy be tried in cases of this kind, especially since no specific therapy is available.

The placental transmission of complement-fixing antibodies from mother to offspring is noted and will be reported more fully later.

Sumario

Histoplasmosis Pulmonar Crónica Complicada por Embarazo y Neumotórax Espántaneo

Preséntase la historia clínica de una mujer de 31 años, de raza blanca, que había tenido histoplasmosis pulmonar reconocida durante ocho años y, durante ese tiempo, tenido cuatro embarazos a término. Con la gestación, la enfermedad tendía a mejorar y, después del parto, las lesiones solían difundirse, las cavernas aumentaban de número y tamaño y la enferma perdía considerable peso. También experimentó dos episodios de neumotórax espontáneo, uno en cada lado, pero se repuso sin complicaciones ni secuelas.

Se discute la larga duración de la dolencia en esta enferma, señalándose la poca información que hay acerca de la evolución a largo plazo y del pronóstico de la histoplasmosis cavitaria crónica.

Ofrécense tres posibles explicaciones de la mejoría de la enferma durante el embarazo, sugiriéndose que se pruebe el neumoperitoneo terapéutico en casos de este género, sobre todo visto que no existe terapéutica específica.

Se apunta la transmisión placentaria de anticuerpos fijadores del complemento de la madre a la prole, dejándose para discusión más completa después.

Resume

Histoplasmose pulmonaire chronique compliquée par la grossesse et deux pneumothorax spontanés

Présentation d'une observation concernant une femme âgée de 31 ans qui présentait une histoplasmose pulmonaire diagnostiquée depuis 8 ans et qui durant cette période avait eu quatre accouchements à terme. Pendant la grossesse, la maladie tendait à s'améliorer, mais habituellement après l'accouchement, les lésions s'étendaient, les excavations augmentaient en nombre et en dimension et la malade perdait beaucoup de poids. Elle avait eu deux pneumothorax spontanés, une fois de chaque côté, mais elle avait guéri sans complication ou séquelles.

La longue durée de la maladie chez cette malade est commentée et la rareté des données relatives à l'évolution à long terme et au pronostic de l'histoplasmose cavitaire chronique est mentionnée.

Les auteurs proposent trois explications possibles de l'amélioration éprouvée par la malade durant la grossesse et suggèrent de traiter ces cas par lá création d'un pneumopéritoine, d'autant plus qu'on ne dispose pas actuellement d'un traitement spécifique.

La transmission placentaire de la mère à l'enfant d'anticorps fixateurs du complément est observée et sera l'objet d'un rapport ultérieur.

Acknowledgments

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PULMONARY ALVEOLAR MICROLITHIASIS[1]

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Pulmonary alveolar microlithiasis is a rare disease of uncertain etiology characterized by calcific intra-alveolar concretions occurring diffusely throughout the lung. The striking roentgenographic appearance is in sharp contrast to the paucity of symptoms early in the disease. The following is the seventeenth reported case of this disease, the fifth to appear in the English literature. It is the eighth case in which the diagnosis was established during life and the seventh in which dust exposure was noted.

Case Report

A 31-year-old Negro was first seen at the Pennsylvania Hospital in October, 1955, complaining of abdominal pain of one day's duration. The pain originated in the right upper quadrant and radiated to the right flank. The patient had enjoyed good health and had no other symptoms.

On examination, the patient was in no distress; the oral temperature was 99.8°F. The epitrochlear, submental, and inguinal lymph nodes were slightly enlarged, nontender, and movable. Medium coarse inspiratory rales were heard throughout the lower portions of both lungs. In the abdomen there was only minimal voluntary muscular guarding in the right upper quadrant. The clinical impression was bilateral basilar pneumonitis with diaphragmatic irritation accounting for the abdominal complaint.

The urine was clear, acid in reaction, had a specific gravity of 1.026, and contained neither reducing substances nor protein. The sediment of the initial urine specimen contained moderate numbers of erythrocytes and leukocytes; subsequent specimens contained neither cell type. There was normal excretion of phenolsulfonphthalein dye. Hemoglobin was 14.0 gm. per 100 ml., hematocrit 52 per cent, leukocytes 14,000 per cu. mm., with normal differential count. Urea nitrogen was 10.0 mg. per 100 ml.; total serum protein was 7.8 gm. with 4.7 gm. of albumin. Serum amylase and lipase activities were within normal limits. The initial electrocardiogram was normal except for transient first-degree heart block which was not present in a subsequent tracing; the electrical axis was normal.

The skin reaction to PPD was negative in strengths of 0.0001 and 0.002 mg.; intradermal tests with histoplasmin and coccidioidin were also negative. Serologic tests for syphilis were negative. Neither routine cultures of the urine nor cultures for tubercle bacilli yielded growth. Concentrates of the small amounts of sputum which the patient was able to produce and of gastric washings were negative for tubercle bacilli on smear and culture.

Roentgenographic studies: A chest film was strikingly abnormal; there were dense infiltrates throughout both lung fields obscuring normal structures and producing a "ground glass" appearance (figure 1). In a film made with the Bucky grid, the pulmonary infiltrates were distributed homogeneously throughout with some sparing of the extreme apices and bases (figure 2). The densities appeared to be of a punctate, calcareous nature. The diagnosis of pulmonary alveolar microlithiasis was suggested both by the senior attending ward physician (Dr. Cooper) and by the radiologist, Dr. Paul A. Bishop. In a plain film of the abdomen, the only abnormality noted was the presence of a few phleboliths in the pelvis. A cholecystogram and an excretory urogram were normal.

Additional occupational history: Further inquiry was made into the patient's past medical and occupational history. It was learned that he had been employed as a grinder in a

[1] From the Division of Medicine and the Ayer Clinical Laboratory of the Pennsylvania Hospital, Philadelphia 7, Pennsylvania.

FIG. 1. Roentgenogram showing fine-grained pulmonary infiltrate throughout both lung fields with some sparing of apices and bases.

brick factory in Augusta, Georgia, in 1941 for a period of one year. The dust concentration in the work area was so heavy that the patient was often unable to see his fellow workers six feet away; he was obliged to clean his clothes with an air jet at the end of the day. No precautions were taken to prevent inhalation of the dust, as it was considered inert. It was learned from the officials of the company that the brick consisted of a kaolin (English china clay) which was fused at high temperature to form the artificial mineral mullite ($3Al_2O_3 \cdot 2SiO_2$) and some fused silica combined in a fine-grained aggregate. It was believed that there was no natural quartz in the fired product and only trace amounts in the clay.

In 1943, the patient had been rejected for military service because of "dust on my lungs." Shortly thereafter he moved to Philadelphia; there has been no further exposure to dust. The patient has remained in good health except for "pneumonia and pleurisy" in 1947, 1950, and 1952; none of these illnesses was severe enough to require hospitalization. The patient was examined by a tuberculosis survey unit in 1953 which reported a photofluorogram as abnormal and suspicious for pulmonary tuberculosis. The patient and his family were followed with periodic chest films, but further studies or treatment were not instituted. These photofluorograms have been reviewed and the changes are similar to those noted in the hospital films.

FIG. 2. The punctate nature of the densities is better seen in a film made with the Bucky grid.

The slight fever and abdominal pain with which the patient entered the hospital subsided promptly with bed rest and chemotherapy. Since discharge, the patient has been working as a stockroom clerk. He complains of dyspnea only on extreme exertion. The basilar rales have persisted.

Pathologic findings: An epitrochlear lymph node biopsy was done; no abnormalities were seen. On the fifth hospital day an intercostal pulmonary biopsy was performed. The visceral pleura was found to be granular, blue-black, and stony hard; the surgeon noted a grating sensation on cutting the lung. A specimen measuring 2 by 1 by 0.7 cm. was obtained.

The biopsy specimen was fixed in Zenker's solution. On microscopic examination, about half of the alveoli contained concentrically laminated concretions which stained deep blue by a hematoxylin-eosin-azure method (figure 3). Some of the concretions distended the alveoli in which they lay. Many of the bodies were fragmented, most of the fracture lines being radial. A foreign-body giant cell response was a prominent feature. These cells, some having as many as 20 regular, vesicular nuclei, often formed part of the structure of a concretion; some were surrounded by calcific lamellae; a few lay free in alveolar spaces (figure 4). Within the cytoplasm of the giant cells round or oval vacuoles, a few asteroid inclusions, and scattered carbon particles were seen. A moderate increase in interstitial fibrous tissue was noted, the alveoli which contained concretions almost uniformly displaying fibrosis of their walls. Scattered small zones of alveolar septal fibrosis were noted elsewhere as well. In addition there was a moderate increase in fibrous tissue about small vessels (figure 5) and in the pleura. A striking feature was the presence of numerous refractile, colorless

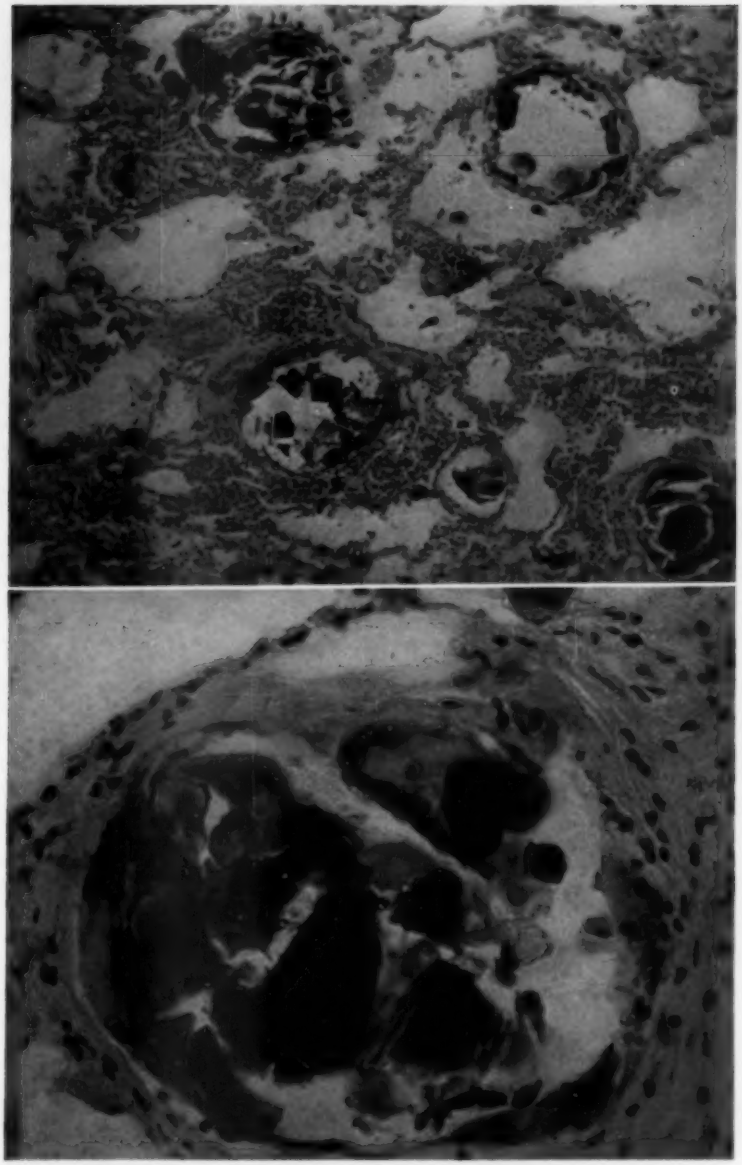

FIG. 3 (Top). Many of the alveoli are occupied by partially fragmented calcific concretions. (60×)

FIG. 4 (Bottom). Giant cells and crystalline particles are seen associated with a laminated concretion. (280×)

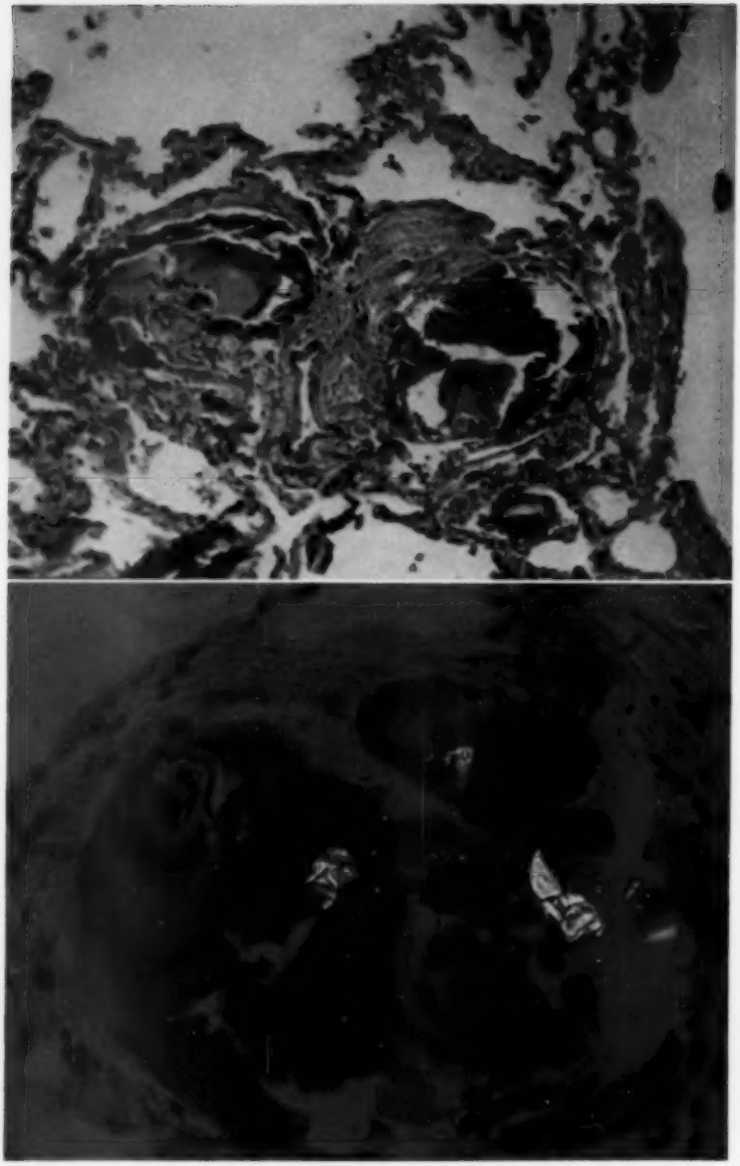

FIG. 5 (Top). Fibrosis about blood vessels and in alveolar walls. (100×)
FIG. 6 (Bottom). Same field as shown in figure 4 under polarized light to demonstrate birefringence in the crystalline particles. (280×)

TABLE 1

PULMONARY FUNCTION STUDIES

Lung Volumes

	Ml.	Per Cent Normal
Inspiratory capacity	1,650	48
Expiratory reserve volume	560	65
Vital capacity	2,180	51
Residual volume	1,590	145
Total lung capacity	3,800	70

Ventilation

	Normal	Air	O_2	7½% CO_2
Respiratory rate (rest)	12/min.	23	23	27
Respiratory depth (rest)	0.5 L.	0.38	0.42	1.24
Respiratory minimal volume	6 L/min.	8.70	9.80	34

Diffusion and Gas Exchange

	Normal	Patient
Diffusing capacity of carbon monoxide	29.5 ml/min/mm Hg	10.2; 10.4; 12.6

Exercise (Treadmill 5% Incline 2 m.p.h. 14 Minutes)

	Respiratory Rate	Minute Volume	Dyspnea
Before	23	8.76	None
After	34	32.00	Moderate

Distribution of Inspired Gas and Blood

Alveolar Gas	Normal	Air
Uniformity	<2.0%	5.2; 8.5; 9.2

Mechanics of Breathing

	Normal	Patient
Maximal expiratory flow rate	400–600 L/min.	338
Maximal inspiratory flow rate	300–500 L/min.	272
Per cent vital capacity in 1″	81%	98.5%
Per cent vital capacity in 3″	95%	100%
Maximal breathing capacity (air)	126 L/min.	154
Lung compliance	0.16–0.22 L/cm H_2O	0.055
Airway resistance	0.5–1.5 cm H_2O/L/sec	1.570
Tissue resistance	0.05–0.35 cm H_2O/L/sec	0.370

TABLE 1—CONTINUED

Arterial Blood

	Normal	Air	O_2	Exercise
Oxygen content (total)	Air—20.6 O_2—22.9 vols%	19.62	22.19*	19.72
Dissolved O_2	Air—0.3 O_2—2.0 vols%	0.30	0.30	0.30
O_2 capacity	20.9 vols%	21.06	21.97	21.97
O_2 saturation	96–99%	91.20	99.60*	88.40
CO_2 content (whole blood)	20.5–23.1 mM/L	17.90	18.20	18
CO_2 content (plasma)	25–28.5 mM/L	21.20	22.20	22
CO_2 tension	38–42 mm Hg	40	43	40
pH	7.38–7.42	7.33	7.30	7.34

* The patient had breathed for only two minutes at the time of this sample.

crystalline particles. These occurred at the periphery of the concretions, between the lamellae of the concretions, within the giant cells, or between the giant cells and calcific lamellae. The crystalline particles were strongly birefringent when viewed with polarized light (figure 6). The concretions were colored deep blue with Mallory's triple connective-tissue stain and blue-black in a Verhoeff-Van Gieson preparation. Elastic fibers could not be demonstrated to participate in the formation of the concretions. The elastic network of the lung was generally well preserved; in only a few areas of fibrosis was there thickening and wrinkling of elastic fibers.

Pulmonary function: Following the pulmonary biopsy, the patient developed a partial right pneumothorax which caused slight dyspnea at bed rest. Except for this instance, he denied ever having had respiratory distress. The results of detailed studies of pulmonary function[2] are listed in table 1. From these studies it was concluded that there was a small total lung capacity owing to restricted inspiratory and vital capacities. Inspired gas was unevenly distributed and gaseous exchange, as measured by the diffusing capacity for carbon monoxide, was definitely impaired. Arterial blood studies indicated moderate oxygen desaturation at rest which was made worse by exercise. The pH and carbon dioxide content of whole blood was low, but there was a normal carbon dioxide tension. These interesting findings suggested a degree of metabolic acidosis presumably due to chronic hypoxia. The over-all tests of mechanical function were normal but the more specific tests indicated a definite reduction in lung compliance, a slightly increased airway, and tissue resistance. The studies indicated that the patient had small, rigid lungs and impaired ability for gaseous exchange.

Calcium metabolic studies: The serum calcium levels were 10.2, 9.8, and 10.4 mg.; serum phosphorus, 3.3 and 3.4 mg. per 100 ml.; serum alkaline phosphatase activity, 5.9, 6.9, and 5.2 Bodansky units. The Sulkowitch test was negative on two occasions and slightly positive once. After four days of restricted calcium intake (137 mg. daily), two twenty-four-hour urine specimens contained 86 and 88 mg. per 100 ml. of calcium. These values are well within the range of normal.

Chemical analysis:[3] A sample of the fused mineral product with which the patient had

[2] Performed at the University of Pennsylvania Hospital through the courtesy of Drs. J. Comroe and R. S. McNeill.

[3] Performed through the courtesy of Dr. G. W. H. Schepers at the Saranac Laboratory.

been working was obtained for analysis. Chemical, petrographic, and X-ray studies indicated that the material was not a clay but a type of synthetic feldspar consisting of approximately 90 per cent anorthite (calcium aluminum silicate) and 10 per cent glass. The results of the chemical analysis, expressed as oxides of the elements, were: silicon 43.9 per cent, aluminum 38.7 per cent, calcium 16.0 per cent, titanium 1.2 per cent, and iron 0.4 per cent.

The quantities of the principal elements in the spectrographic analysis of the remaining pulmonary tissue, also expressed as oxides of the elements, were: phosphorus 60.0 per cent, calcium 24.0 per cent, silicon 5.2 per cent, chromium 5.3 per cent, iron 1.7 per cent, magnesium 1.47 per cent, aluminum 0.66 per cent. The chromium content may be attributed to previous fixation in Zenker's fluid.

Comment

The designation, "pulmonary alveolar microlithiasis," was first applied by Puhr (1) in 1933 to describe a case in which the alveoli were filled with laminated, calcified concretions. Pertinent data regarding cases which conform morphologically to Puhr's case and to the present one are assembled in table 2. It is to be noted that ten cases occurred in males and seven in females. The diagnosis has been made at autopsy in nine cases, by pulmonary biopsy in four cases, and by the roentgenographic and clinical features in five cases.

The ages at the time of death in the nine autopsied patients ranged from twenty-eight to seventy-two years, six of the deaths occurring between the ages of forty-one and fifty-four. In all of the autopsied cases but those of Sharp and Danino (2) and of Schildknecht (3), the pulmonary disease was thought to have been the cause of death. In the former case there was a moderate degree of mitral stenosis and a terminal bronchopneumonia; in the latter case, the patient was a paretic who succumbed after a course of malarial therapy, the pulmonary disease having been asymptomatic. The case of Harbitz (4) is difficult to evaluate inasmuch as the author stated that the concretions were interstitial in position, but the single low-power photomicrograph resembles pulmonary alveolar microlithiasis. The four cases reported by Mikhailov (5) are of especial interest inasmuch as three of the cases occurred in a family group. Cases No. 11, 12, and 13 of this series had slightly abnormal tests of liver function; a biopsy specimen of the liver in Case 11 contained nonspecific granulomata.

Intra-alveolar pulmonary concretions have long been recognized in the lung in two forms other than the microliths under discussion. The pathogenesis of none of these concretions has been established and, because of morphologic, clinical, and roentgenographic dissimilarities, it seems best to consider them separately.

Corpora amylacea in the lung were first described by Friedreich in 1856 (6). They are rounded intra-alveolar bodies which may be laminated and which may or may not stain as amyloid (7). They are not extensively calcified and are not visible roentgenographically. They are found in areas of emphysema, atelectasis, chronic pneumonitis, and infarction (6–8). They are not productive of pulmonary disease, but are, rather, a manifestation of another disease. Their origin, whether from alveolar lining cells (7) or from inspissation (9) is uncertain. Giant cells may be associated with corpora amylacea, but it is not certain whether they are fundamental in the formation of the concretion or whether they are secondary to the presence of the concretion as a foreign body. Cases of pulmonary alveolar microlithiasis differ from those in which numerous corpora amylacea occur in that antecedent pulmonary disease is lacking in the former group, except possibly for the case of Sharp and Danino (2) in which mitral stenosis was present.

TABLE 2

CASES WHICH CONFORM MORPHOLOGICALLY TO CASE HEREIN REPORTED

Case	Date	Author	Sex	Age	Diagnosis	Dust Exposure
1	1918	Harbitz (4)	F	41	Autopsy	
2	1932	Schildknecht (3)	F	45	Autopsy	
3	1933	Puhr (1)	F	49	Autopsy	
4	1947	Mariani *et al.* (26)	M	28	Autopsy	Stonemason
5	1949	Leicher (27)	M	44	Autopsy	Farmer
6	1950	Bénard *et al.* (23)	M	72	Autopsy	Stonemason
7	1951	Lindig (28)	M	40	Roentgenogram	Rock driller
8	1953	Sharp and Danino (2)	F	54	Autopsy	
9	1954	Manz (29)	F	37	Autopsy	Clay dust
10	1954	Petrányi and Zsebök (30)	M	13	Biopsy	
11	1954	Mikhailov (5)	F	15	Roentgenogram	
12	1954	Mikhailov (5)	F	32	Biopsy	
13	1954	Mikhailov (5)	M	40	Roentgenogram	
14	1954	Mikhailov (5)	M	17	Roentgenogram	
15	1955	Badger *et al.* (15)	M	54	Autopsy	
16	1955	Kent *et al.* (16)	M	35	Biopsy	Rock and fiber dust
17	1956	Present case	M	31	Biopsy	Clay dust

Case	Tissue Analysis	Clinical Features	Additional Findings
1	Ca-PO_4 and CO_3	Dyspnea, cyanosis, cardiac decompensation	Cor pulmonale
2	Ca-PO_4 and CO_3	Asymptomatic	Dementia paralytica
3	Ca-PO_4	Pulmonary insufficiency	Cor pulmonale
4	Ca-CO_3 and PO_4	Dyspnea, cyanosis, polycythemia	Cor pulmonale
5	Ca-PO_4 and CO_3	Dyspnea	Digital clubbing
6	Ca-CO_3	Pulmonary edema	Birefringent lipid present
7		Decreased vital capacity	Living after 6 years
8	Ca-PO_4	Asymptomatic	Moderate mitral stenosis, bronchopneumonia
9	Ca, Al, Mg-CO_3	Cardiac decompensation	Pneumonia in childhood
10		Asymptomatic	Presumably living
11		Cough	Clubbed fingers
12	Ca-CO_3 and PO_4	Dyspnea, cyanosis	Clubbed fingers
13		Slight dyspnea	Clubbed fingers. Brother of patient 12
14		Asymptomatic	Son of patient 13
15	Ca-PO_4 and CO_3	Pulmonary insufficiency	Cor pulmonale, nephrolithiasis
16		Asymptomatic	Birefringent lipid present. Patient living
17	Ca-PO_4	Decreased vital capacity	Patient living

Nodular pulmonary calcification and ossification are known to occur in mitral stenosis. The distribution is similar to that seen in microlithiasis, with the roentgenographic densities appearing principally in the lower lung fields, sparing the extreme apices and bases. The nodules, however, tend to be larger and less numerous than in microlithiasis, ranging up to as much as 2 cm. in diameter. In these cases, ossification is a prominent feature, whereas it is only occasionally encountered in microlithiasis. In reported cases of pulmonary calcification in mitral stenosis (10–13), formation of masses of bone with crenated edges, occupying several alveolar spaces, was noted. Interstitial calcification has also been noted. The case described by Friedreich (14) in his second report has been mentioned as an example of microlithiasis, but the concretions were transparent, not calcified; they were thought to consist largely of iron salts and were associated with tight mitral stenosis. In view of the profound changes in hemodynamics and structure known to occur in the "mitral lung," it seems best to consider pulmonary calcification and ossification in this disease separately both from corpora amylacea and from microlithiasis. There is no doubt, however, that corpora amylacea may occur in the lung of a patient with mitral stenosis (14) or that a patient with morphologically acceptable microlithiasis may have mitral stenosis (2). The pathogenesis of pulmonary calcification and ossification in mitral stenosis is uncertain. Some (11) maintain an origin from organization of a specific rheumatic pneumonia, while others (10, 13) believe that organization of congestive hemorrhages may be responsible.

An abnormality of local calcium tissue metabolism in the lung has been postulated in microlithiasis (15, 16). No defect in systemic calcium metabolism has been demonstrated although such a defect is suggested in Cases 11, 12, and 13 listed in table 2 in which serum calcium levels were elevated. The pattern of calcification conforms neither to that of dystrophic calcification nor to that of Virchow's metastatic calcification (17). The precipitation of calcium salts in the lung would seem to be facilitated by the increase in pH of venous blood as a result of loss of carbonic acid. Why precipitation should occur in the lung, however, and not at other sites of anion excretion (such as the stomach and kidneys) would be difficult to explain. The presence of renal calculi in Badger's patient (15) is of interest in this regard.

The relationship of microlithiasis to dust exposure deserves consideration inasmuch as exposure to mineral or fiber dust has occurred in six cases. In another case, the patient was a farmer and may have been exposed to dust. It is tempting to try to relate the formation of microliths in the present case to inhalation of either kaolin or dust from the finished product. A few cases have been reported in which pneumoconiosis has occurred as a result of inhalation of kaolin or fuller's earth (18–21). In the first three of these cases there was fine nodulation in the interstitial tissues of the lung, having predominantly lymphatic distribution. In the fourth case, there were conglomerate fibrotic nodules in the upper lobes. None of these cases resembles the present one. The possibility cannot be denied that the patient may have had pulmonary microlithiasis before exposure to dust in 1941.

The history of dust exposure, however, in six of the reported cases seems more than merely coincidental. The results of the chemical analyses do not directly support this hypothesis. There are two difficulties. The first is that the brick was stated by the management of the plant to have been an aluminum silicate, whereas the analysis indicates that it is a calcium aluminum silicate. Second, the amounts of aluminum and silicon obtained on spectrographic analysis of the fixed tissue are within normal limits for an adult, only calcium and phosphorus being significantly increased. Although aluminum silicate is apparently not a significant component of the concretions, a dust-caused etiology has not

been excluded. The dust may have provoked tissue damage and subsequently have been removed; this process is known to occur in the case of certain amorphous silica dust exposures (22). Additional support is given to a dust-caused etiology by the occurrence of birefringent crystalline particles in the concretions in the present case, although the possibility exists that the crystals may have resulted from chemical combination of the constituents of the concretions with those of the fixative. Similar particles have not been described in other cases, although "Maltese cross" birefringence due to lipid has been noted in the concretions by others (16, 23). No material was available in the present case for study of lipid content.

Although the diagnosis of pulmonary microlithiasis has been most commonly made at autopsy, there are distinctive clinical and roentgenographic features by which the diagnosis can be established during life. The most outstanding feature early in the disease is the paucity of symptoms in sharp contrast to the strikingly abnormal chest film. Terminally, progressive pulmonary insufficiency, cor pulmonale, and congestive heart failure appear. The roentgenographic appearance is characteristic, with fine-grained pulmonary infiltrates involving the lungs diffusely, with some sparing of the extreme apices and bases. The differential diagnosis, from the roentgenographic point of view, involves exclusion of pulmonary calcification in mitral stenosis and the so-called "benign pneumoconioses." The former may be excluded by the absence of the usual clinical, electrocardiographic, and roentgenographic features of that disease. The latter consist of a number of occupational diseases (stannosis, siderosis, baritosis, kaolinosis) in which mineral dusts accumulate in the lung (24, 25), producing a similar roentgenographic appearance but in which significant pulmonary fibrosis and pulmonary disability are thought not to occur. These conditions may be excluded on the basis of the occupational history and also of pulmonary biopsy.

Summary

A case of pulmonary alveolar microlithiasis, in which the diagnosis was established during life by means of pulmonary biopsy, has been presented. The patient was a healthy Negro male who gave a history of short but heavy exposure to mineral dust. The diagnosis was suspected clinically because of the strikingly abnormal roentgenogram and the paucity of clinical findings. In addition, a review of the literature on the subject has been presented with special emphasis on the possible relationship of this disease to dust exposure and its differentiation from other diseases exhibiting intra-alveolar pulmonary concretions.

Sumario

Microlitiasis Alveolar Pulmonar

Preséntase un caso de microlitiasis alveolar del pulmón, en el que se estableció el diagnóstico durante la vida del enfermo por medio de la biopsia pulmonar. Tratábase de un varón sano de raza negra, que dió una historia de exposición breve, pero intensa, a polvo mineral. Se sospechó el diagnóstico clínicamente debido a la notable anormalidad de la radiografía y a la escasez de hallazgos clínicos. Discútense la posible relación de esta dolencia con la exposición al polvo y su diferenciación de otras enfermedades que manifiestan concreciones intraalveolares en el pulmón.

Resume

Microlithiase alvéolaire du poumon

Présentation d'un cas de microlithiase alvéolaire du poumon, dont le diagnostic fut obtenu par biopsie pulmonaire chez un malade. Le sujet était un Nègre en bon état général

dont les antécédents révélaient une exposition brève, mais intense, aux poussières minérales. Le diagnostic avait été suggéré cliniquement par la radiographie, qui était extraordinairement anormale, et la rareté des signes cliniques. Une corrélation possible de cette affection et l'exposition aux poussières et sa différenciation d'autres affections comportant des concrétions pulmonaires intra-alvéolaires est étudiée.

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ANAPHYLAXIS TO VIOMYCIN[1]

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The purpose of this report is to present a case of anaphylaxis which occurred during the course of viomycin therapy in a child being treated for chronic pulmonary tuberculosis. There are many references in the literature dealing with the various toxic manifestations of this antituberculous drug. However, toxic effects involving the vasomotor system have been described as minor, with one exception. Lemming and Mellender (1) have reported a case in which anaphylaxis to viomycin occurred in a forty-year old man. The following is the only case which has come to the writer's attention in the United States or British literature in which such a severe reaction of hypersensitivity has occurred.

Case Report

The patient, a twelve-year-old girl of Puerto Rican extraction, was admitted to Bellevue Hospital on January 4, 1955. Her symptoms began three months prior to admission, in Puerto Rico, where she had had a single episode of hemoptysis followed by a persistent productive cough, low-grade fever, and generalized indisposition. On admission, the temperature was 99.4°F.; pulse, 90 per minute; respiration, 20 per minute; and blood pressure was 90 systolic and 60 diastolic in mm. of mercury. Positive physical findings were limited to the chest. Bronchial breathing and medium moist rales were heard over the first three left anterior interspaces. Bronchophony and whispered pectoriloquy were present over the same area. The chest roentgenogram revealed clouding of the upper left lung field, with a cavity in the area between the first and second anterior interspaces. On the right there was a dense area of clouding through the second anterior interspace. Several areas of calcification were seen on the right, at the apex, root, and cardiophrenic angle. A diagnosis of far advanced chronic pulmonary tuberculosis was made, and antituberculous therapy (isoniazid, 5 mg. per kg., and PAS, 12.0 gm. per day) was instituted on January 7, 1955.

The PAS therapy was omitted on January 17, 1955, when a generalized pruritic, papulovesicular eruption appeared which was thought to be due to this drug. The eruption cleared promptly and on January 22, 1955, a sulfone (Promizole®) was added in place of PAS.

The patient's general condition was good, she began to gain weight, and the chest roentgenogram remained substantially unchanged. By May 13, 1955, eight consecutive gastric cultures had been reported positive for tubercle bacilli. At this time, PAS was cautiously added to the therapeutic regimen (isoniazid and sulfone) with no untoward reaction. On May 31, 1955, the patient's organisms were reported partially resistant to isoniazid (5.0 γ per ml.) and susceptible to streptomycin. The isoniazid dosage was increased to 10 mg. per kg., streptomycin (1.0 gm. per day) was begun, PAS was continued, and the sulfone was omitted.

Chest roentgenograms and tomograms of September 19, 1955, showed a cavity opening into the bronchus of the anterior segment, left upper lobe, with another cavity in the second left anterior interspace. In the upper portion of the right upper lobe several small

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areas of calcification were present, with some fibrosis and evidence suggestive of bronchogenic "spill." Lateral tomograms suggested involvement of the left lower lobe. The gastric washings continued to show tubercle bacilli, with large numbers of colonies on culture. The patient's clinical course, however, was one of gradual improvement.

By January 6, 1956, with the chest roentgenogram substantially unchanged, drug-susceptibility studies revealed that the patient's organisms were totally resistant to streptomycin (1,000 γ per ml.) and were still partially resistant to isoniazid (5.0 γ per ml.). In consultation with the chest surgeons, it was decided that surgical resection of the caseous focus in the left upper lobe was indicated in this pre-pubescent girl. After one year of therapy, a good deal of unresolved tuberculous disease remained, the gastric cultures were persistently positive for tubercle bacilli, which had demonstrably high resistance to one major drug and partial resistance to another. Accordingly, in preparation for surgery, on January 6, 1956, daily therapy with viomycin (20 mg. per kg.) and pyrazinamide (35 mg. per kg.) was instituted; isoniazid, streptomycin, and PAS were omitted.

On February 6, 1956, a left upper lobectomy was performed. The left upper lobe was small, fibrotic, nonaerating, and was densely adherent to the parietal pleura. Palpation of the lower lobe revealed many small (3 to 4 mm.) nodules in the apical segment and scattered nodules of similar size in the basilar segments. The immediate postoperative course was essentially benign. Pyrazinamide was continued daily and viomycin was reduced on February 25, 1956, to 1.0 gm. twice weekly.

On the morning of February 25, 1956, less than five minutes after the patient had received her usual biweekly injection of viomycin intramuscularly, she developed an acute generalized allergic response, characterized by dyspnea, difficulty in phonation, pallor, giddiness, extreme weakness, a fall in blood pressure, and a generalized giant urticarial eruption. The patient was immediately given an antihistaminic (Benadryl®) intravenously and, in addition, the usual symptomatic treatment for allergic shock. There was a prompt amelioration of all of the severe symptoms, although the weakness and urticaria persisted in a gradually diminishing degree for several hours. The date of the injection preceding the one which caused the reaction was February 22.

In an effort to identify the offending allergen, 0.1 ml. of a solution containing one part of viomycin and thirty-nine parts of distilled water was injected intradermally into the volar aspect of each forearm. The viomycin used in the test on the right arm came from the same vial used at the time of the reaction, and the viomycin used in the left arm came from a vial with an entirely different lot number. Scratch tests, done prior to the intradermal tests, had given equivocal results. Large wheals and local erythema developed immediately at the site of both intradermal inocula, followed by the appearance of giant pseudopodia and intense local itching. Two hours later, a fine pruritic eruption developed over the extensor surfaces of the extremities which persisted for twelve hours. Several other patients on the ward who got viomycin injections from the same vial on the same day as the patient of the present report had no local or general signs or symptoms, which would seem to offer adequate proof that the drug was not a primary irritant.

On March 1, 1956, the administration of cycloserine (1.0 gm. per day), isoniazid (10 mg. per kg.), and sulfone (Promizole) was instituted in place of the previous therapy of viomycin and pyrazinamide. By June 15, 1956, the patient continued to show clinical improvement on this regimen and is still being followed on the pediatric chest ward. Gastric cultures have been negative for tubercle bacilli since December 27, 1955.

Comment

In the case described by Lemming and Mellender, the patient had received biweekly viomycin without incident for one month. There was an unintentional interruption of therapy for eighteen days, owing to difficulty in obtaining the drug. The anaphylactic reaction occurred with the first injection following the eighteen-day interval. Similarly, in another of their patients being treated with viomycin at this time, who also had had

therapy suspended for eighteen days, a minor allergic reaction (urticaria and tachycardia) developed one week after viomycin therapy was resumed. On the basis of their experience, they concluded that one should avoid interruptions in viomycin therapy of longer than two to three days. In the present case, the patient had been on biweekly viomycin for one month, without interruption, when the reaction occurred.

Viomycin exerts an appreciable effect on *Mycobacterium tuberculosis* (2–6). While it is generally considered to be therapeutically inferior to either isoniazid or streptomycin (2, 3, 7), it is effective in the therapy of disease caused by isoniazid- and streptomycin-resistant organisms (2, 4, 7, 8). The clinical toxicity of viomycin is greater than that of streptomycin (8, 9). It exerts a toxic effect on the eighth nerve similar to streptomycin and, in addition, its toxic manifestations are seen affecting the kidney, electrolyte balance, the vasomotor system, and the hemopoetic system (2, 3, 5–7, 10).

It is not within the scope of this paper to discuss the details of viomycin toxicity nor is it intended to disparage the use of this drug in the therapy of tuberculosis. It is intended to report a severe allergic response which occurred incidental to the use of viomycin and to encourage a certain awareness of *all* of the toxic potential of this drug.

Summary

Viomycin is a valuable antituberculous drug whose toxic side-effects are well documented in the literature. A case of anaphylaxis during the course of viomycin therapy is reported which occurred in a child with chronic pulmonary tuberculosis whose bacilli had become resistant to isoniazid and streptomycin.

Sumario

Anafilaxis a la Viomicina

La viomicina es una valiosa droga antituberculosa cuyos efectos colaterales tóxicos se hallan bien documentados en la literatura. Descríbese un caso de anafilaxis en el transcurso de la viomicinoterapia que se presentó en un niño con tuberculosis pulmonar crónica cuyos bacilos se habían vuelto resistentes a la isoniacida y la estreptomicina.

Resume

Anaphylaxie à la Viomycine

La viomycine est un médicament antituberculeux de valeur dont les effets toxiques secondaires sont mis en évidence dans de nombreuses publications. L'auteur rapporte un cas d'anaphylaxie survenue au cours de la viomycinothérapie chez un enfant atteint de tuberculose pulmonaire chronique et dont les bacilles étaient devenus résistant à l'isoniazide et à la streptomycine.

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Notes

SOLARIZED ROENTGENOGRAPHIC DUPLICATION

The reproduction of roentgenograms has always been a problem and presents many aspects of the light effect on partially exposed film that are poorly understood. No reference can be found in the literature which presents satisfactory end results using standardized simple procedures available in a department of radiology.

Three different methods have been tried over a period of the past nine years. Much of the information obtained was through trial and error so that now an acceptable duplicated roentgenogram can be obtained from the original film without too much repetition and experimentation.

The need for film reproduction arose in the radiology department because of the necessity of obtaining material for teaching purposes. The intent was to have a set of representative films of interesting cases on hand in the department at all times for visual teaching purposes. Quite frequently an interesting set of roentgenograms would become lost, misplaced, or transferred to another hospital upon the patient's hospital discharge.

The first method employed was the use of actual sunlight for the source of the photographic solarized effect. Outdated unexposed films were used for the duplication process. The unexposed film was removed from the black paper folder and subjected to the light present in a room or was held in the sunlight for approximately two minutes. This served as an introductory agent to produce film crystal instability prior to the actual reproduction procedure.

Let it be assumed that a 14- by 17-inch film was to be reproduced. The preliminarily solarized film was placed upon a sheet of glass over which was placed the film to be reproduced. The glass had to be clear, symmetrical, and without blemish. A second 14- by 17-inch sheet of glass served as a cover. These were stabilized upon a sheet of Masonite® so that contact was not disturbed during the second two-minute sunlight exposure period. This method did not prove to be completely satisfactory as the solarization process was dependent upon the time of day, presence of obstructions to the sunlight and, also, the season of the year. Furthermore, there was no control over the density and contrast to be obtained on the second film.

A second method was devised which utilized a General Electric Photoflood No. 2 lamp placed in a 12-inch diameter, round reflector. The steps of the procedure are much the same as those noted above. The outdated unexposed film was removed from the jacket and subjected to room light for approximately two minutes. This procedure was carried out in regular room light or window light. Part of the two-minute period of preliminary film exposure to room light was used in assembling the various materials. These steps were observed by the interval timer. A sheet of Masonite plus a sheet of double-thickness window glass were placed upon a table. Upon these, in succession, were placed a smooth, white, 14- by 17-inch, intensifying screen, the preliminarily solarized film, and then the roentgenogram to be duplicated. This was covered by the second sheet of double-thickness window glass. The light source to film distance was 40 inches. The assembled items were then ready for the second light exposure.

Approximately fourteen to fifteen minutes of light exposure is needed to reproduce a chest roentgenogram and approximately ten to eleven minutes to duplicate a pyelographic examination. The exposed film is taken to the processing room and developed under conditions similar to those for any other roentgenogram except that the proc-

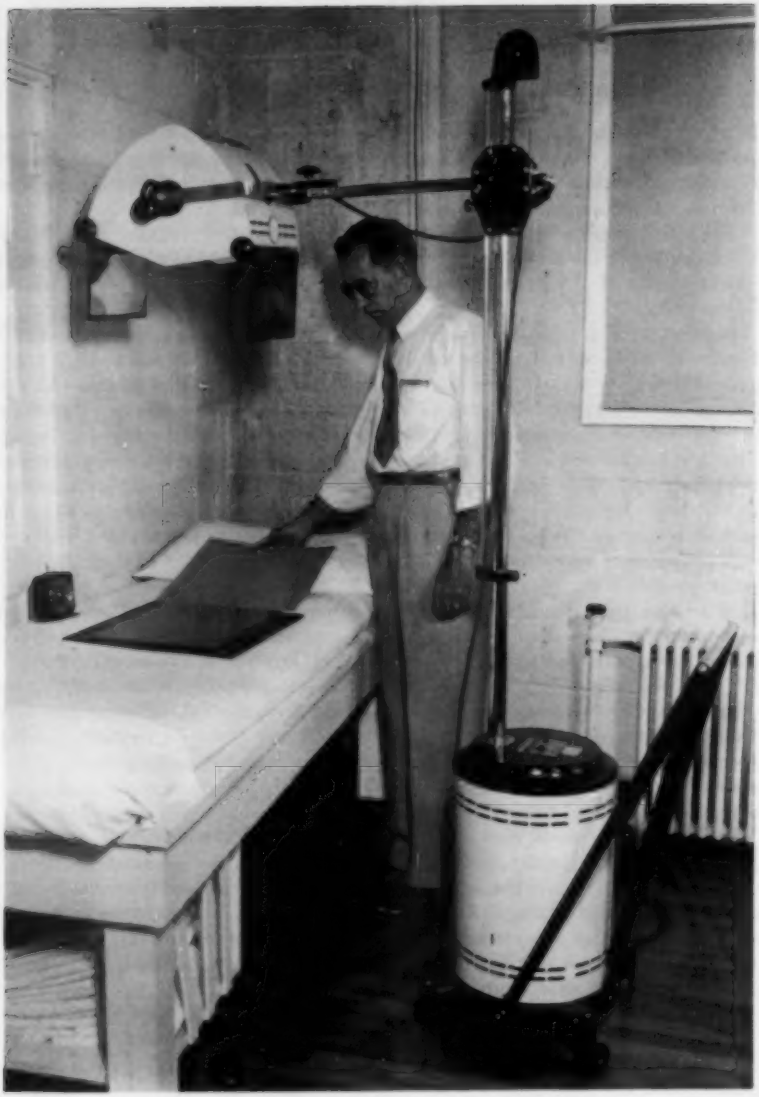


FIG. 1. The ultraviolet therapeutic unit, set up with the necessary accessories in a room where the procedure can be accomplished without interruption. The operator wears a pair of protective goggles and is shown removing the top cardboard cover for the desired time exposure.

ess is not carried out in the usual darkness. This allows a better direct film image visualization as it appears in the developing solution. When the image begins to appear, which is usually within one minute, it is immediately washed and placed in the fixing bath. The technician, after suitable experience, can predetermine the desired contrast and depth of density which should result in a satisfactory film for interpretation.

The third method, which is currently used, has yielded satisfactory end results. A Hanovia super-Alpine, therapeutic, mercury arc, air-cooled, mobile, 110 volt, 60 cycle, ultraviolet lamp is the light source (figure 1).

The steps in this procedure are much the same as those previously described.

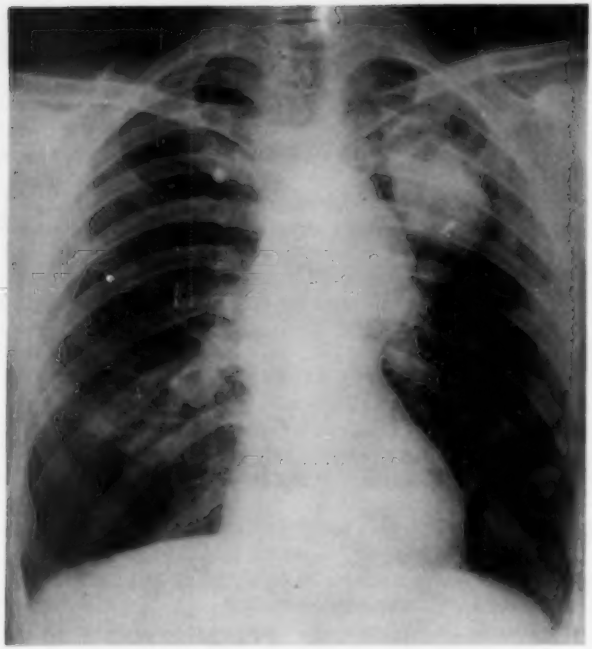
An outdated film is obtained and subjected to a preliminary period of solarization. This can be obtained by one of three means. The film can remain either in the room light or sunlight for approximately two minutes. As an alternative, the film can be subjected to an X-ray exposure of $\frac{1}{20}$ second, 30 milliamperes, and 40 kilovolts. It has been found that this latter method is simpler and causes fewer artifacts with much more ease of film handling. Since the ultraviolet lamp has to be in operation for approximately five minutes to gain its full output, a cardboard cover is applied to the top of the complete assemblage of the parts needed for the exposure.

The parts, as assembled, listed from below upwards (figure 1) are as follows: a sheet of Masonite or plywood, a piece of double-thickness window glass, intensifying screen, the unexposed film which has been solarized initially, the film to be duplicated, a second piece of double-thickness window glass, and the cardboard cover. The size of all of these parts is 14 by 17 inches. The double-thickness window glass was chosen because it affords better contact and is devoid of imperfect undulations of either contact surface.

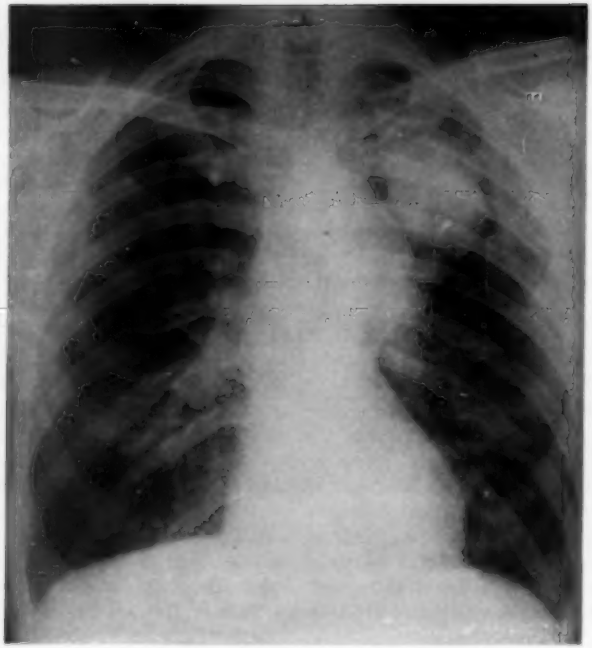
* * *

The physical and chemical reasons for accomplishing this duplicating process are not understood. Suffice it to say that, despite this, the end results have been satisfactory. In general, it can be said that the outdated film must possess crystals which are susceptible to this procedure. The initial solarized film is apparently temporarily placed in a positive state by the preliminary exposure to a light source. This source of radiant energy may be incandescent light, sunlight, or X-rays. It is thought that this initial energy must create an instability of the emulsion film substance which makes the crystals sensitive to the second exposure of light. As a result of the second exposure to light, a negative film is obtained which responds to the usual developing used in every radiology laboratory. It has been observed that occasionally a positive duplicate film would be obtained. The circumstances under which this would result were: a lengthened initial period for solarization, a foreshortened period for the second light exposure, and lengthened developing time in the dark room.

In general, it can be stated that, the longer the time of solarization, the lighter will be the resulting duplicated film. The reverse corollary of this is true, namely, that, if the film to be duplicated is light, then a longer secondary exposure time will cause it to be darker. This is a distinct advantage in that quite frequently the resulting reproduced film has many qualities which are superior to those of the original film. The time-exposure range for using the ultraviolet light source is from fifteen seconds to two minutes. The average exposure time needed to solarize the usual postero-anterior roentgenogram of the chest or an extremity is twenty to thirty seconds. This is similarly true for a roentgenogram of a skull or a plain film of the abdomen which is of average normal contrast for roentgenographic interpretation. If the skull or plain film of the abdomen is a little lighter than usual, the time can be reduced to fifteen seconds or, if it is a little darker, it can be increased to forty-five seconds.



a



b

FIG. 2*a*. Original roentgenogram showing carcinoma of the lung.
b. Duplicate film showing carcinoma of the lung.

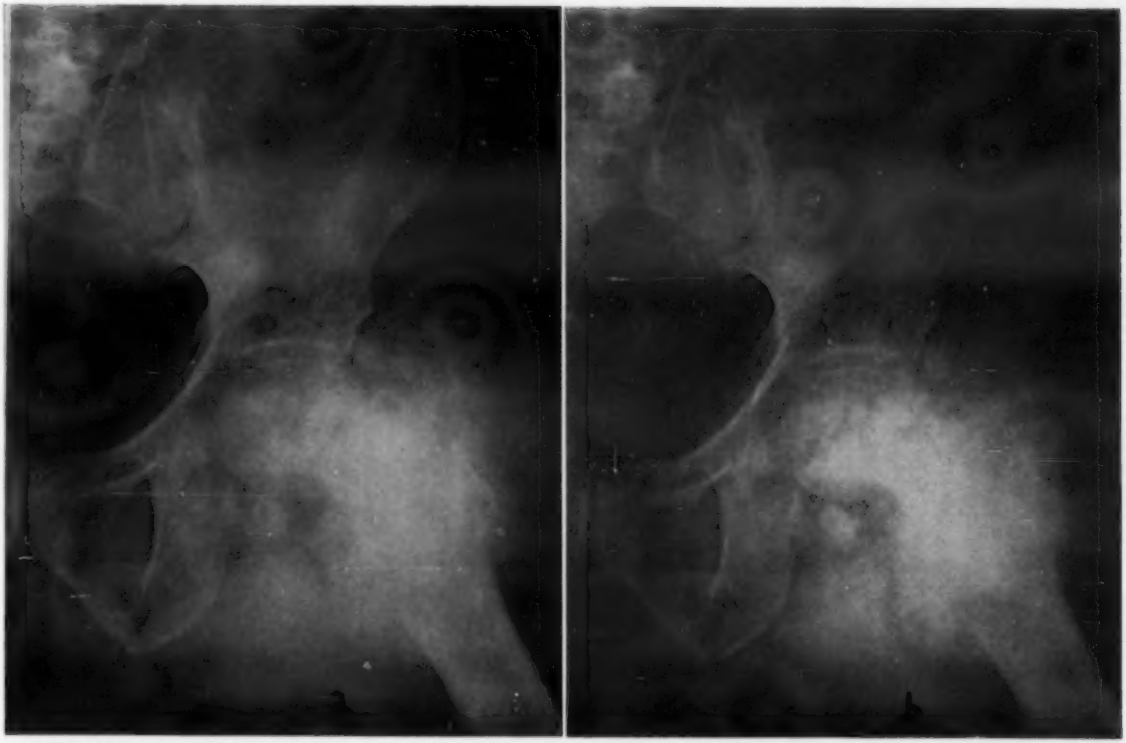


FIG. 3. Roentgenograms of the left hip reveal an osseous sarcoma of the proximal left femur. (*a* is the original; *b*, the duplicate.)

Two illustrative duplicated films are presented with the original roentgenograms. An example of carcinoma of the lung is illustrated in figure 2, original and duplicate photographs, respectively (*a* and *b*). In figure 3 an osseous sarcoma of the proximal left femur is revealed. Figure 3*a* is the original; *b* is the duplicate.

Three methods of producing roentgenographic duplication are presented. The solarized process, depending upon an incandescent light source or sunlight, has been tried and replaced by the third method which utilizes an ultraviolet lamp. This latter procedure is not difficult and does not consume very much time. Any department of radiology can set aside a room with the various needed parts, and with very little training one of the members of the technical staff can produce satisfactory duplicate films. It has been proved to be a distinct advantage at this hospital for teaching purposes. No knowledge is claimed for the physical backgrounds of this procedure. The comments and suggestions given are those which have been obtained over a period of nine years, and the various techniques as given have been gained from a long period of trial and error.

The parts and equipment which are needed to carry out this reproductive technique are presented above, and are demonstrated in figure 1.

The salient features for accomplishing this process are:

Possession of old, outdated, although not exposed, roentgenographic films. It is preferable that the expiration date be augmented to at least two years.

The films which are to be used for solarization need a preliminary exposure to radiant energy. This can be obtained by a two-minute exposure to room light, actual sunlight, or to a short exposure of X-rays.

The actual reproduction or solarization process can best be accomplished by the use of an ultraviolet lamp. The procedure is simple and the exposure times are short.

Various component parts as assembled to produce the solarized exposure are listed. Smooth surfaces, removal of debris, firm contact, and immobilization are the main essentials needed to produce a satisfactory second film. When using the ultraviolet lamp, the complete assemblage must be protected by a sheet of cardboard since the lamp cannot be disconnected between succeeding exposures.

Strict adherence to the exposure and developing times should result in satisfactorily duplicated films without very much experimentation.

In summary: A method has been outlined to obtain duplicate films of satisfactory roentgenograms. Occasionally the produced duplicate film can excel the original. With very little experience a technician who works in the smallest of laboratories can become adept at this procedure. A reproduced film can be used as a substitute for the original. This is especially applicable for a laboratory where teaching is performed. Not uncommonly an interesting roentgenogram may be lost or the need to send it to another location may arise, especially if the patient moves or is transferred by employment. This duplication procedure can thus be of aid to any practitioner of the various medical specialties.

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June 20, 1956

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(5) Nichols, B. H., and Root, J. C.: Radiology, 1936, *27*, 362.

LYMPHATIC DRAINAGE OF THE PLEURAL SPACE IN DOGS, AS DETERMINED BY STUDIES WITH RADIOACTIVE GOLD (AU^{198})

Certain manifestations of the distribution of extrapulmonic tuberculous lesions have been related to the pattern of lymphatic drainage in the involved area. In particular, the lymphatic drainage of the pleura in tuberculous pleuritis has been believed to be of importance in the pathogenesis of at least two types of lesions: anterior chest wall cold abscesses and fistulas, secondary to caseous destruction of an internal mammary lymph node; and Pott's disease, secondary to caseous destruction of a para-aortic lymph node with caries of the adjacent vertebra by direct extension.[1] There is experimental and clinical evidence that these groups of lymph nodes do receive lymphatic vessels draining the parietal pleura. Very little has been written concerning pleural lymphatic drainage into other groups of lymph nodes, particularly the tracheobronchial nodes.

In the course of some experimental work concerning the pleural lymphatic drainage in relation to bronchogenic carcinoma, data were obtained which indicate that a considerable amount of the pleural lymphatic drainage may be into the tracheobronchial nodes in dogs.

In a series of 10 dogs subjected to left pneumonectomy, radioactive gold (Au^{198}) was placed into the empty hemithorax in doses of 40 to 70 millicuries. The Au^{198} was administered in the immediate postoperative period, and the animals were sacrificed at intervals up to twenty-two weeks. Histologic sections and radio-autographs of the tracheobronchial nodes revealed the presence of the Au^{198} therein together with varying degrees of radiation destruction of the nodes.[2] A second series of dogs was subjected to pneumonectomy plus instillation of tracer doses (2 to 4 millicuries) of Au^{198} and the dogs were sacrificed at intervals up to forty-eight hours. Examination of the tracheobronchial nodes by a counting technique revealed the presence of significant amounts of radiation in relation to other tissues examined.[2]

A third series of 9 dogs was then studied to ascertain the distribution via lymphatics of Au^{198} instilled into the pleural space of intact dogs. The results are reported herein.

Adult mongrel dogs weighing 10 to 15 kg. were used. Anesthesia was accomplished by intravenous pentobarbital. Lung inflation was maintained with endotracheal intubation and positive pressure, with a Burns valve. A one-inch incision was made over the sixth interspace; the parietal pleura was exposed, and a pinprick opening was made therein. Radioactive gold colloid[3] in doses of 2 to 4 millicuries was injected in a volume of 4 ml. using a length of small polyethylene tubing. During this maneuver a few cubic centimeters of air might enter the pleura and be immediately aspirated; the incision was closed tightly without further air leakage. At forty-eight hours the animal was sacrificed and an autopsy performed. Lymph nodes and other tissue biopsies were obtained as indicated in table 1. During autopsy the treated hemithorax was entered last, and the only specimens possibly altered by surface contamination were the lung biopsies, paravertebral (or azygos on the right) nodes, and retrosternal nodes on the involved side. In dog *No. 9* there was a connecting pleural cavity, whereas in the others there was a transparent membrane mediastinum. This was fortunate, for most dogs have connecting pleural spaces. In addition, for all

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[2] Data to be published.

[3] Used as Aurcoloid, a radioactive gold (Au^{198}) colloid manufactured by Abbott Laboratories, the particles of which are approximately 0.003 microns in diameter. Half-life is 2.69 days, and the radiation consists primarily of a 0.97 Mev beta ray and a 0.411 Mev gamma component.

TABLE 1

Dog Number	1	2	3	4	5	6	7	8	9
Au^{198} Dose Millicuries	2	2	2	2	4	2	2	4	2
Side Administered	Right	Right	Right	Right	Right	Left	Left	Left	Left
Tissue Counts									
Right cervical node	0.000203	0.000519	0.000217	0.000244	0.000293	0.00254	0.00088	0.00337	0.00621
		0.175	0.000241						
Left cervical node	0.000242	A*	0.000136	0.000108	0.00271	0.000036	0.00165	A	0.00348
			0.000202			0.0007	0.000389		
Right axillary node	0.00235	0.00132	0.000348	0.000728	A	0.0012	1.96	0.00084	0.0039
Left axillary node	0.000165	A	0.000237	0.000643	0.02	0.0263	0.00292	0.00205	0.00354
					0.00267				
(Right) azygos node	A	10.9	827	A	A	A	A	A	A
Left paravertebral node	A	6.01	A	1,890	A	579	A	A	A
Right retrosternal node	228	56.4	377	861	535	897	694	596	1,426
Left retrosternal node	71.8	0.00205	457	1,671	701	857	386	194	1,321
Right lumbar node	16.61	0.00098	0.000347	496	0.00286	15.1	0.00226	200	0.00184
Left lumbar node	0.00226	0.00245	0.442	A	0.00267	26.4	0.00226	A	A
Portal node	0.00171	0.00413	0.122	0.00113	0.00405	0.00231	0.00296	0.00369	A
	0.04057								
	0.00177								
Splenic node	0.0863	0.00154	1,541	346	0.038	0.131	0.011	0.212	0.0431
Right paratracheal node	0.0604	61.7	A	177	163	50	397	453	A
					1.15				
Left paratracheal node	25.3	0.01	8.97	880	A	222	57.5	15.5	102
Anterior tracheal node	0.00962	883	A	14.9	A	1.46	98.5	0.0128	330
Right main bronchial node	0.0503	0.0186	0.000744	0.00625	1.69	0.0312	0.132	0.0246	0.201
Left main bronchial node	0.00357	7.03	9.59	0.0769	0.0947	0.487	1.13	0.159	A
Subcarinal node	0.0329	A	2.25	2.76	1.58	4.97	9.57	0.544	0.118
Right lung biopsy	N†	0.0117	1.56	0.16	N	N	N	N	N
Left lung biopsy	0.0184	N	N	N	0.126	0.0202	0.0604	0.171	0.166

* A = Tissue absent in animal.

† N = Not examined.

Tissue concentrations of radiogold in various tissueg 48 hours after intrapleural administration of Au^{198}. Results are expressed as microcuries per gram of tissue weight, correction being made for isotope decay.

In some instances more than one lymph node was found in the given location and these were individually counted.

animals, in order to remove as much surface contamination as possible, each tissue was dipped in three washings of saline and the excess saline was removed by blotting. Tissues were weighed wet on a Roller-Smith balance. Radioactivity was determined with a gamma-sensitive well counter of known counting efficiency, and expressed as microcuries per gram of wet tissue.

The results of tissue count are presented in table 1. The figures for the cervical and axillary nodes can be taken as maximal for intravenous contamination, if such occurred, in the incision. The lung biopsy figures probably can be taken as maximal for the surface contamination source of error. The azygos, paravertebral, and restrosternal nodes, as expected, showed the highest counts. The nodes around the main bronchus showed counts which were questionably, if at all, elevated. The anterior tracheal nodes and paratracheal nodes, however, showed significantly elevated counts in most instances. The subcarinal nodes showed slightly elevated counts. Elevated counts appeared sporadically elsewhere, as in the lumbar and splenic nodes.

* * *

The present data, plus the results of other investigations carried out in this and other laboratories,[4] suggest the possibility that the parietal pleural lymphatic vessels may

[4] MATUSKA, R. A., *et al.*: J. Thoracic Surg., 1955, *30*, 525.

drain into the tracheobronchial lymph nodes, although to a lesser extent than into the sternal and vertebral groups. It is possible that the drainage into the tracheobronchial nodes is primarily from the mediastinal parietal pleura, but this is not known. It is also possible that the intrapleural Au^{198} is picked up by the visceral subpleural lymphatic channels and carried to the tracheobronchial nodes. This could be only a partial explanation of the present data, inasmuch as rapid pickup of Au^{198} by tracheobronchial nodes can be demonstrated in acute experiments in animals subjected to pneumonectomy.

In summary: Au^{198} administered intrapleurally to dogs was taken up primarily by retrosternal and paravertebral lymph nodes, and to a lesser extent by tracheobronchial lymph nodes.

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DETECTION OF MYCOBACTERIUM TUBERCULOSIS IN SPUTUM BY PEPSIN DIGESTION AND INTERFACE CONCENTRATION WITH PENTANE[1]

Digestion with alkali or acid, followed by centrifugation, is employed in most laboratories for processing sputum for the detection and isolation of tubercle bacilli. These procedures are not without harmful effects on the tubercle bacilli.[2-6]

The use of proteolytic enzymes as sputum digestants has been suggested by various workers.[7-13] For a number of years hydrocarbons and other water-insoluble fat solvents have been suggested as concentrating agents for tubercle bacilli.[14-21] For one reason or another the use of enzymes for digestion and hydrocarbons for concentration has not been widely adopted by diagnostic laboratories for the detection of tubercle bacilli in sputum.

By the use of pepsin for digestion and pentane for concentration, a procedure has been developed for the detection and isolation of tubercle bacilli from sputum which has given significantly better results in this laboratory than the widely used trisodium phosphate-centrifugation procedure.

Selection of enzyme digestant: Of the several proteolytic enzymes tested, trypsin and pepsin appeared to be the least toxic and most efficient. At first trypsin seemed the enzyme of choice until it was discovered that, on inoculation of the Löwenstein-Jensen medium, sufficient enzyme was transferred to cause digestion of the slants. For this reason pepsin was substituted since it is largely inactive at the pH of the isolation medium. At the optimal pH, most sputum specimens are digested by 0.2-0.4 per cent pepsin (1:10,000) within two hours at 37°C.

Pepsin shows optimal activity at approximately pH 2. Several acids were tested in various concentrations to determine which was most suitable for activating the enzyme, which was least toxic for the tubercle bacilli, and which had most buffering capacity in the sputum. The toxicity of hydrochloric acid for tubercle bacilli has been frequently

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[1] Abstract of dissertation presented by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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TABLE 1

CITRIC ACID AND PEPSIN COMBINATIONS FOR BEST DIGESTION OF SPUTUM

	Citric Acid			
	0.5%	1.0%	1.5%	2.0%
Pepsin:				
0.1%	−	−	+/−	+
0.25%	+/−	+	+	+
0.30%	+	+	+	+
0.50%	+	+	+	+

− = incomplete digestion
+ = complete digestion

referred to in the literature.[25-27] Of the several inorganic and organic acids tested, citric acid appeared to be most ideal. A concentration of 2 per cent citric acid showed little toxicity for *M. tuberculosis* H37Rv and fully activated the pepsin. It is also a good acid buffer, and the addition of sputum causes little change of pH.

To determine the optimal concentrations of pepsin and citric acid for digestion of sputum, tubes of various conbinations were made and a clot of pooled sputum was added to each tube. The tubes were incubated at 37°C. for two hours and the results are recorded in table 1. The lowest concentration of citric acid and pepsin which digested the sputum in two hours may be seen from the table to be 1.0 per cent and 0.25 per cent, respectively. A digestant with 2 per cent citric acid ($C_6H_8O_7 \cdot 2H_2O$) and 0.5 per cent pepsin (1:10,000) was therefore adopted. This digestant is used in a 1:1 ratio with sputum.

Selection of concentrating agent: Eighteen organic fat solvents were selected for testing as concentrating agents, namely: *n*-amyl alcohol, iso amyl alcohol, butyl acetate, toluene, hexone, carbon tetrachloride, monochlorobenzene, 2-chloro-2 methyl benzene, isoprene, diacetyl, methyl formate, methylal, 3-chloropropene, propylene oxide, *n*-pentane, paracymene, alpha terpeneol, and terpinolene. Preliminary experiments showed the best results with pentane and terpinolene, i.e., low toxicity and high concentration of tubercle bacilli at the interface.

Further experiments showed pentane to be preferable to terpinolene, being less toxic to tubercle bacilli and easier to handle. Pentane has a boiling point of 36.3°C. and specific gravity of 0.63. When shaken with water it separates quickly and sharply. Being highly volatile it evaporates readily from culture media and slides. It appears to have no toxicity for tubercle bacilli. For other bacteria the toxicity is variable, but the results show a considerable reduction in total sputum flora which grow out on blood plates after exposure to pentane for one-half hour.

To show the efficiency of pentane as a concentrating agent for tubercle bacilli, the following experiments are presented:

To 10 ml. of tuberculous sputum digest 1 ml. of pentane was added and thoroughly agitated. This was poured into a burette and allowed to stand one-half hour. The lower third was allowed to run into a receiving tube; the middle third, into a second tube; the top third

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TABLE 2

DISTRIBUTION OF ACID-FAST BACILLI AND OTHER BACTERIA AT PENTANE-WATER INTERFACE AND AQUEOUS PHASE

		Interface	Aqueous Phase		
			Top Third	Middle Third	Lower Third
Burette	Acid-fast bacilli	Many	None	None	None
	Contaminants	Few	Many	Many	Many
Frozen tube	Acid-fast bacilli	Many	None	None	None
	Contaminants	Few	Many	Many	Many

(under the interface), into a third tube; the interface and pentane, into a fourth tube. The contents of each tube were stained and the numbers of acid fast-bacilli and other bacteria recorded.

One milliliter of pentane was added to 10 ml. of tuberculous sputum digest in a 150 by 15 mm. tube and was thoroughly agitated. After one-half hour the contents of the tube were frozen by exposure to −20°C. The frozen plug was removed by brief immersion in hot water and divided into four portions as in experiment 1, after which the melted portions were stained.

The results of these experiments are recorded in table 2, which shows that the tubercle bacilli are concentrated at the interface while the other bacteria are not.

Pepsin-pentane procedure for concentrating tubercle bacilli: The digestant was an aqueous solution containing 0.5 per cent pepsin (1:10,000) and 2 per cent citric acid ($C_6H_8O_7 \cdot 2H_2O$). The solution was allowed to stand overnight and was sterilized by filtration through a Seitz filter. (It may be stored in a refrigerator without loss of activity for several months.)

Pentane is highly volatile and inflammable. A Bunsen burner is unnecessary throughout the procedure.

For digestion apparatus, large culture tubes (75 ml.) were found to be suitable. They were covered with small beakers or aluminum caps which are safer than cotton plugs. For agitation of the pentane with the digested sputum a 5 ml. pipette fitted with a 3 ml. rubber bulb was used. When many specimens are to be processed an assembly consisting of a 50 ml. compression bulb, a 12-inch piece of rubber tubing, and a 5 ml. pipette was found to be practicable and less tiresome.

N/1 KOH and brom thymol blue indicator (0.04 per cent): The brom thymol blue indicator is dissolved in alcohol and adjusted to a green color. The concentrations of the KOH and indicator need not be accurate.

The recommended procedure is as follows:

Add an approximately equal volume of digestant to the sputum. Mix and transfer mixture to digestion tube. The tube should not be more than half full.

Incubate the digestion tube at 37°C. With most sputum specimens two hours are sufficient. Large shreds or particles which may appear undigested usually disintegrate when agitated.

Add a few drops of brom thymol blue and sufficient KOH to give the digest a light green color.

Add 3 to 4 ml. of pentane (to give about an inch layer) to the digestion tube. With the pipette and bulb vigorously mix the pentane and sputum digest. Do this for approximately two minutes. The objective is to bathe the tubercle bacilli with pentane.

Let stand for ten to fifteen minutes for separation of the two phases.

Aspirate the interface for slide and culture. The pentane need not be removed. The little pentane carried along with the inoculum will not matter since it is highly volatile. The same pipette may be used throughout the procedure for one specimen.

A total of 212 sputum specimens from local sources was examined for the presence of tubercle bacilli by the pepsin-pentane method and by the trisodium phosphate-centrifugation method. Some of the sputum specimens were from patients with undiagnosed chest conditions; others were from known tuberculous patients under treatment. The sputum specimen was divided as equally as possible for processing with each of the two methods. In the phosphate method 10 per cent Na_3PO_4 was mixed with an equal volume of sputum and incubated for two hours at 37°C. The mixture was then transferred to a 15 ml. tube and centrifuged at approximately 2,000 r.p.m. in an angle centrifuge for fifteen minutes.

Duplicate tubes of Löwenstein-Jensen medium were inoculated with appropriate material from each concentration method and incubated at 37°C. From the same materials slides were prepared for acid-fast staining. Material from both procedures was placed on the same slide for simultaneous staining.

TABLE 3

COMPARATIVE EFFICIENCY OF Na_3PO_4 METHOD *versus* PEPSIN-PENTANE METHOD FOR THE ISOLATION OF TUBERCLE BACILLI FROM SPUTUM

Incubation in Days	Positive Cultures							
	Only T	Only P	T and P	Total	Total T	Total P	T Per Cent	P Per Cent
14	4	24	40	68	44	64	65	94
21	6	28	57	91	63	85	69	93
23	8	25	65	98	73	90	74	92
35	11	26	74	111	85	100	76	90

T = 10% Na_3PO_4 procedure
P = pepsin-pentane procedure

TABLE 4

COMPARATIVE EFFICIENCY OF PEPSIN-PENTANE AND 10 PER CENT Na_3PO_4 Procedures for the Detection of Tubercle Bacilli in Sputum by Staining and Culture

Culture After 35 Days' Incubation	Acid-Fast Stain				Totals
	P+, T+	P+, T−	P−, T+	P−, T−	
P+, T+	49	11	5	9	74
P+, T−	11	4	2	9	26
P−, T+	3	4	1	3	11
P−, T−	11	17	1	72	101
Totals	74	36	9	93	212

P = pepsin-pentane procedure
T = 10% Na_3PO_4 procedure
+ Indicates that typical acid-fast bacilli were found on the slide and that typical growth was observed on the Löwenstein-Jensen slants.
− Indicates absence of typical acid-fast bacilli by stain and absence of typical growth on culture.

The results of the comparative study of isolation efficiency are given in table 3.

The data in table 3 show not only a higher percentage of isolations by the pepsin-pentane method compared with the phosphate-centrifugation method, but a much earlier appearance of colonies of tubercle bacilli in the tubes inoculated from the pepsin-pentane concentrate. Statistical analysis shows the difference in percentage isolations to be significant.

The data in table 4 summarize the results obtained with all specimens by the culture method and by staining. The superiority of the pepsin-pentane method over the phosphate method is again quite striking. Of the 212 specimens examined, 110 (52 per cent) were positive by staining from the pepsin-pentane interface and 83 (39 per cent) from the phosphate centrifugation. This difference is statistically significant. The slightly higher percentage of positive results obtained by staining (56 per cent) compared with culture (52 per cent) may be due to the large number of sputum specimens from patients receiving chemotherapy.

In summary: A procedure has been described for the detection of tubercle bacilli in sputum. The specimen of sputum is first liquefied by treatment for two hours with a mixture of pepsin and citric acid. The liquefied sputum is then thoroughly agitated with pentane. The two phases are allowed to separate and the material at the pentane-water interface is removed for staining and culture. A comparative study is reported of the efficiency of the above method and the conventional sodium phosphate-centrifugation method using 212 sputum specimens. The results show the pepsin-pentane method to be definitely superior to the phosphate method by giving a higher percentage of positive acid-fast stains, a higher percentage of positive cultures on Löwenstein-Jensen medium, and earlier detectable growth on the Löwenstein-Jensen medium.

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GENITOURINARY TRANSMISSION OF TUBERCULOSIS[1]

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

When men with genitourinary tuberculosis have asked us if there was danger of infecting their wives, we have said "No."

We began to wonder about the truth of this answer when we recovered tubercle bacilli on culture from a patient's semen in 1952, but dismissed this finding as a laboratory error. However, like most dismissals of tubercle bacilli on similar grounds, it continued to haunt us.

In April, 1954, an article appeared by Lattimer and associates[2] on the "Transmission of Genital Tuberculosis from Husband to Wife via the Semen." They reviewed the eight cases in the literature (mostly European) and added one of their own.

This made the possibility of genital transmission interesting but, out of the thousands of cases of genitourinary tuberculosis, did not seem to make this form of transmission a major public health problem; nor did we feel justified in preaching an abstinence which we felt might not be observed strictly when the nights were balmy and the moon was full.

In February, 1954, a patient had been admitted to our Sanatorium with a typical left tuberculous epididymitis and innocent of any aspirations to provide some further information on an obscure medical problem. He was a 29-year-old recent Italian immigrant, who gave a history of fatigue and blood-spitting in Italy in 1946, but supposedly without demonstrably significant changes in his chest roentgenogram at that time.

His chest film on admission to our Institution showed some obliteration of the left costophrenic angle and scattered calcifications in both apices and hilar areas. He had obtained 30 gm. of streptomycin from the United States in 1946 and was treated with 0.5 gm. daily for sixty days at that time. His symptoms disappeared and he remained quite well until a mass developed in the left scrotum in December, 1953. On admission to a general hospital, tubercle bacilli were found in pus aspirated from the mass.

Following admission to the Sanatorium, urine specimens were negative on smear and culture for tubercle bacilli, but intravenous and retrograde pyelograms showed a small cavity in the lower calyx of the right kidney. Unfortunately the semen was not examined at first. He was started on streptomycin-PAS therapy in February, and no semen was examined until May. At that time, and on all subsequent examinations, it was negative on smear and culture for tubercle bacilli. His prostate was within normal limits.

Our principal interest in the case developed in December, 1954, when his wife was admitted with a mass in the right groin, from which tubercle bacilli were recovered. She was 19 years of age on admission and had also come from Italy, where her mother may have had active pulmonary tuberculosis some years ago. In October, 1954, she noticed the mass for the first time. Her tuberculin test was positive, but there was no evidence of any active pulmonary disease. She was married in June, 1953. Their only child was born in May, 1954. She had two positive cultures from urine obtained in January, 1955. Pyelograms have been normal, and we suspect that the tubercle bacilli came from the lower genital tract rather than from the kidneys. On triple antituberculous drugs the mass has subsided to the size of a normal inguinal lymph node.

We believed that this sequence of events was strongly suggestive of genital transmission

[1] The cases included were first reported at the Ontario Laennec Society in November, 1955.

[2] LATTIMER, J. K., *et al.*: Am. Rev. Tubere., 1954, *69*, 618.

and were just considering tightening our regulation of the patients' extramural activities when the second apparent instance was seen.

On January 3, 1955, a 19-year-old male German immigrant was admitted to the Sanatorium after large numbers of tubercle bacilli had been demonstrated in his urine and pyelography in one of the general hospitals had shown the right kidney to be destroyed. He had had one attack of gross hematuria almost eighteen months before, but this had cleared and he had been free of symptoms until about the summer of 1954, when he began to have frequency, and pain at the end of micturition. These symptoms increased markedly in severity, and he had another frank hemorrhage in the fall of 1954. On admission to the Sanatorium, urine was positive on smear and culture for tubercle bacilli, and urinalysis showed many pus cells, some erythrocytes, some bacteria, and 1- to 2-plus albuminuria. His prostate contained a definite hard nodule in its upper part, just to the right of the median furrow, felt quite readily on rectal examination. There was no evidence of any old or recent epididymitis. His renal tuberculosis has been treated with triple-drug therapy and nephrectomy.

His wife, an 18-year-old Canadian, was admitted to the Sanatorium on January 30, 1955, with a tuberculous inguinal lymphadenitis. They had been married in December, 1953. Their only child was born in May, 1954, after an entirely normal labor and delivery. Early in the summer of 1954, she first noted a mass in the left groin. When the supposed "abscess" was opened by her family physician, it failed to heal and continued to discharge. When she was seen in our outpatient department as a contact of her husband, her urine was also examined and was found to contain organisms resembling tubercle bacilli on smear. Her tuberculin test was strongly positive. The discharging lymphadenitis was considered to be a tuberculous process. After admission, pyelography revealed no evidence of any kidney lesion, and urine obtained at cystoscopy was negative on smear and culture.

Although several 24-hour specimens of urine were positive for tubercle bacilli, we do not think that these came from the urinary tract but rather were contaminants from a lesion probably in or about the vagina. Unfortunately a careful pelvic examination was not done at that time. One examination of material from the discharging inguinal lymph node showed tubercle bacilli on direct smear. Prior to the patient's admission, treatment with penicillin had produced no change whatever in the process, but on triple antituberculous drugs, the lesion has healed, and we have found no more acid-fast organisms. It may be of some interest to point out that, after she and her husband were on leave from the Sanatorium for one day in May, she became pregnant again.

It is quite impossible, in either of these cases, to estimate the time between the wife's exposure and the development of the lymphadenopathy, but Lattimer believes that this may be as little as three to four weeks.

In both of these males, there was an extremely probable source of tubercle bacilli in the genital tract, along with their urinary tuberculosis, although no tubercle bacilli could be isolated from the semen. Although the patients stated that the ejaculate was normal, the semen was definitely reduced in volume to 1.0 ml. or less. This agrees with the findings of Lattimer. However, in neither case was there any evidence whatever of any reduced potency.

In both of these females, tubercle bacilli were found in 24-hour specimens of urine without any evidence of any urinary tract disease. We believe that they came from the lower genital tract. If this possibility is not kept in mind, incorrect diagnoses may be made, and the husband's genital tuberculosis may go unsuspected.

We hope that these findings will emphasize the possibility of venereal infection with

tuberculosis. Although such infection is not common, our experience suggests that it may be more common than we had supposed. As so often happens, more evidence of this form of transmission may be found if we look for it.

The condition is of importance, not only from the standpoint of tuberculosis control, but also because it gives rise in the female to an inguinal lymphadenopathy which may be confused with other diseases, including those of venereal origin.

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EDGAR T. PEER[2]

March, 1956

GROWTH REQUIREMENTS OF ISONIAZID-RESISTANT TUBERCLE BACILLI

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

Since the publication of some of our observations relating to the growth requirements of isoniazid-resistant tubercle bacilli (Cohn, M. L., *et al.*: Am. Rev. Tuberc.,1954, *70*, 641; 854), somewhat contradictory studies on the same problem by other investigators have appeared in the REVIEW. It is apparent to us that we may not have described our observations and interpretations clearly enough.

Peizer *et al.* (Am. Rev. Tuberc., 1956, *74*, 428) recently presented the results of some experiments which they interpreted as indicating that "Carbon dioxide is not an essential requirement for growth of tubercle bacilli when egg-yolk or serum media are employed." The materials and methods which they employed in arriving at this conclusion show that they automatically excluded the possibility of discovering strains of tubercle bacilli which require a higher concentration of carbon dioxide for growth *in vitro* than the great majority of strains. As sources of inoculum for their study, they only used subcultures of strains which grew well *without* added carbon dioxide on primary isolation from tuberculous patients. Furthermore, we take this opportunity to state that none of our observations relative to the carbon dioxide requirements for growth of tubercle bacilli have indicated that such requirements are specifically related to isoniazid resistance or to the manifestation of isoniazid resistance.

The studies of Barry *et al.* (Am. Rev. Tuberc., 1955, *71*, 785) also deserve some comment. These investigators clearly demonstrated that different strains of isoniazid-resistant tubercle bacilli have different growth requirements. They, therefore, concluded by implication that it was unlikely that one hypothesis could be devised to explain those growth requirements of isoniazid-resistant tubercle bacilli which are specifically related to their property of isoniazid resistance. It is not commonly appreciated that different isoniazid-susceptible "wild" strains of tubercle bacilli differ widely in their requirements for optimal growth. Unless these differences are taken into account, it is evident that the elucidation of growth requirements specifically related to isoniazid resistance would appear to be more complicated than they actually are.

Previous studies from our laboratories have shown that those isoniazid-resistant mutants which are completely deficient in catalase require catalase or agents having catalase activity for optimal growth *in vitro*. Such strains probably have this requirement because of their hypersusceptibility to hydrogen peroxide produced during their cultivation *in vitro* and must have some outside help to protect them against this very toxic product.

[2] Our thanks to Dr. C. G. Shaver, Superintendent of Niagara Peninsula Sanatorium, for permission to publish this material.

Extension of these studies, both at the research and at the routine clinical laboratory levels, has confirmed this conclusion. Indeed, the techniques of isolation of tubercle bacilli from sputum, as performed in our laboratories (Middlebrook *et al.*: Am. Rev. Tuberc., 1954, *70*, 854), have permitted us to cultivate every strain encountered in patients admitted to the National Jewish Hospital during the past two years. In other words, since 1953 no patient has been encountered who yielded specimens which were repeatedly positive on smear but negative on culture. In more than 95 per cent of cases this has been accomplished by incubation of cultures no longer than three weeks before reading: some 5 per cent or less of patients harbor strains which yield no growth, or only microscopically demonstrable growth, in twenty-one days and require thirty to thirty-five days for growth to grossly visible colony size. Many of these latter strains from patients who had not previously been treated with isoniazid were catalase positive. Thus, it would appear that, when special precautions are taken to protect tubercle bacilli from endogenous and exogenous hydrogen peroxide, the isoniazid-resistant catalase-deficient strains manifest no more complicated requirements for growth *in vitro* than do the wild strains.

This is not to say that the growth requirements of tubercle bacilli are not "complicated," because even in our studies the culture medium employed is not completely defined—it contains two ill-defined components: serum albumin (Fraction V) and agar-agar. Also, it is important to state that the application of our methods and culture media has been of no avail in solving the problem of the smear-positive, culture-negative specimens from *closed* necrotic lesions. Like other investigators, we have encountered a considerable incidence of smear-positive, culture-negative, surgically resected specimens; all of these were from patients whose sputum had yielded consistently positive cultures up to the time of sputum conversion, which occurred some months prior to surgery. On the other hand, it is equally important to point out that, during the same two-year period, more than 95 per cent of smear-positive materials from *open* cavitary lesions removed surgically have proved to be positive on culture. These experiences are related primarily to emphasize, on the one hand, that the growth requirements of tubercle bacilli in general are indeed complicated but, on the other hand, that the specific growth requirements relevant to isoniazid resistance and the commonly associated catalase deficiency constitute a well-defined phenomenon with an obvious metabolic explanation.

In view of the fact that most routine clinical laboratories do not use the oleic-acid–albumin agar type of culture medium for primary isolation of tubercle bacilli, we wish to point out that the simple expedient of adding a few drops of a sterile 10 to 20 γ per ml. solution of crude beef liver catalase to the surface of the common egg media, before inoculation, provides adequately for the catalase requirements of catalase-deficient strains. This practice does not interfere with the results of drug-susceptibility (or -resistance) tests, nor does it obscure the results of catalase tests on colonies which develop on the medium.

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NOTICES

SIXTH BIENNIAL POST-GRADUATE COURSE ON DISEASES OF THE CHEST

The Sixth Biennial Post-Graduate Course on Diseases of the Chest, co-sponsored by the Los Angeles County Tuberculosis and Health Association and the Los Angeles County Medical Association's Section on Diseases of the Chest, will be given on January 31, February 1 and 2, 1957, in Los Angeles, California. The course will be a practical presentation of the current status of chest diseases with special emphasis on diagnosis and treatment. Topics will include diagnostic procedures, emphysema, pneumonia, pleural diseases, pulmonary tuberculosis, carcinoma of the lung, pulmonary function tests, fungal diseases, cor pulmonale, surgery of the heart, and surgery of the esophagus. Included in the program will be a Cancer Workshop, with division of the class into small discussion groups involving a panel composed of a surgeon, an internist, and a pathologist.

The faculty will include David T. Carr, M.D., Mayo Clinic, Rochester, Minnesota; Maurice S. Segal, M.D., Boston; Edgar W. Davis, M.D., Washington, D. C.; H. Corwin Hinshaw, M.D., San Francisco; and local chest specialists.

The registration fee of $35.00 will include a banquet on Thursday evening. Further information and advance registration may be obtained by writing to: David Salkin, M.D., 1670 Beverly Boulevard, Los Angeles 26, California.

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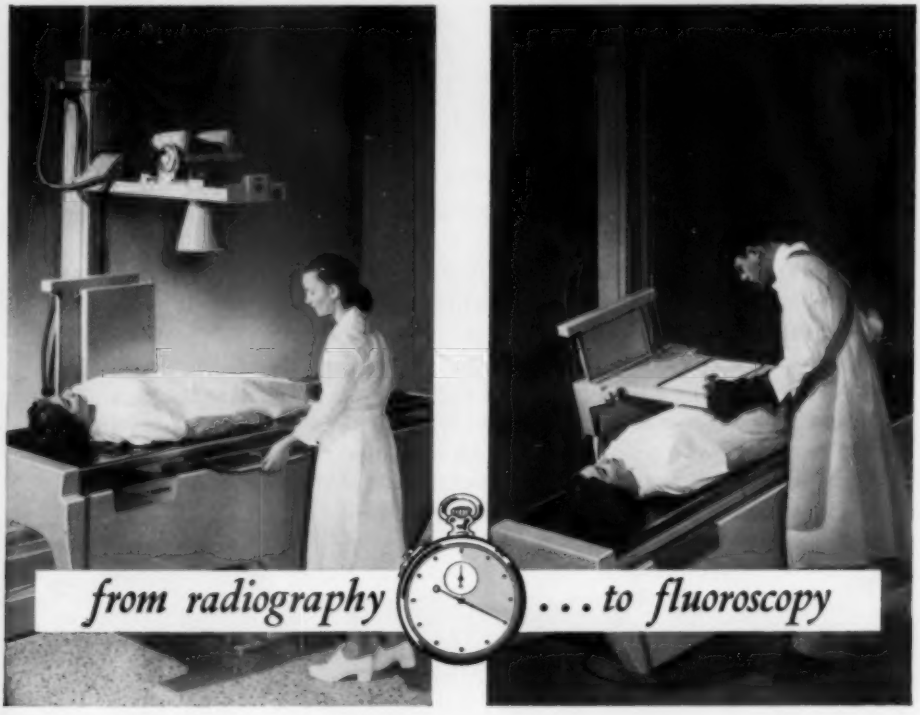

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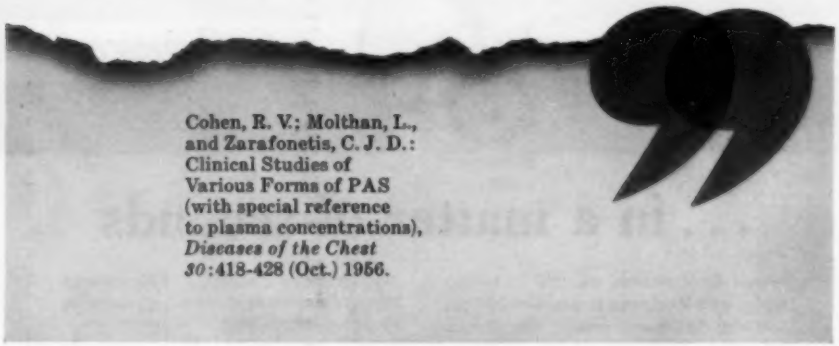
It is concluded that KPAS is superior to other forms of PAS.



Cohen, R. V.; Molthan, L., and Zarafonetis, C. J. D.: Clinical Studies of Various Forms of PAS (with special reference to plasma concentrations), *Diseases of the Chest* *30*:418-428 (Oct.) 1956.

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